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O. Appel

Am 12. September 1937 starb völlig unerwartet infolge eines Herzschlages in Quedlinburg a. Harz der Phytopathologe Dr. Fritz Sattler.

Er war am 3. Juni 1905 in Ulm a. d. Donau geboren. Von Jugend auf von einer leidenschaftlichen Liebe zu allem, was mit Botanik zusammenhing erfüllt, bahnte er sich unter den allergrössten Schwierigkeiten und persönlichen Opfern den Weg zu seiner Lieblingswissenschaft. Er studierte von 1925 bis 1931 in Tübingen und Freiburg Naturwissenschaften. Im Jahr 1932 begann er in Bonn am Institut für Pflanzenkrankheiten seine Promotionsarbeit über "Thilavia basicola" und führte gleichzeitig Untersuchungen über Viruskrankheiten und deren Übertragung durch Blattläuse aus. Im Jahre 1934 kam er nach Giessen an die Hauptstelle für Pflanzenschutz. Hier beendete er seine Promotionsarbeit und beschäftige sich vorwiegend mit Nematodenkrankheiten der Rüben (*Heterodera Schachtii*), Kohlfliege (*Chortophila brassicae*), Brennfleckenkrankheit an Bohnen (*Gloeosporium Lindemuthianum*), Löffelkrankheit an Azaleen (*Exobasidium japonicum*). Im Frühjahr 1935 legte er nach seiner Doktorpromation als 1. die in Giessen neueingerichtete Prüfung für Pflanzenschutz (Pflanzenarzt) ab.

Danach bearbeitete er im Auftrag des Reichsforschungsdienstes der Landbauwissenschaften an der Abteilung für Pflanzenkrankheiten des Institutes für Pflanzenbau und Pflanzenzüchtung der Ludwigs-Universität, Giessen die Biologie und Bekämpfungsmöglichkeiten des Apfelblütenstechers (*Anthonomus pomorum*). 1937 konnte er bereits seine umfrangreichen Versuche, die sich über ganz Süddeutschland erstreckten mit Erfolg abschliessen.

Im Frühjahr 1937 trat er als Phytopathologe und Saatzuchtleiter in die Saatzuchtfirma Gebr. Dippe A. G. in Quedlinburg ein. Dort beabsichtigte er auch seine Arbeit über Anthonomus pomorum zu vollenden und sie als Habilitationsschrift einzureichen.

Die Gediegenheit seines Wissens, der nie rastende Fleiss, seine ernste Auffassung wissenschaftlicher Arbeit liessen hoffen, dass Sattler bald mit weiteren Arbeiten hervortreten und Tüchtiges leisten würde. Diesen Hoffnungen hat sein Tod ein zu frühes Ziel gesetzt.

Dr. Sattler veröffentlichte:

Zur Biologie von *Thielavia basicola* (Zopf); Phytopathologische Zeitschrift Bd. 10, Heft 1, Jahrgang 1936.

Erneutes Massenauftreten der Runkelrübenmotte (*Phthorimaea ocellatella* Boyd) in Hessen; Nachrichtenblatt für den deutschen Pflanzenschutzdienst.

Der Apfelblütenstecher (*Anthonomus pomorum* L.), vorläufige Mitteilung über die Versuchsjahre 1935 und 1936; n. a. veröffentlicht im "Obstbau" Heft 1, 1937.

Das Ergebnis seiner erfolgreich beendeten Untersuchungen über den Apfelblütenstecher erscheint als vorläufige Mitteilung z. Zt. und der ausführliche Bericht in der Zeitschrift "Der Forschungsdienst" im Laufe des Jahres durch seinen Nachfolger und seine technische Assistentin.

FACTORS INFLUENCING THE PATHOGENICITY OF PYTHIUM DE BARYANUM ON SUGAR BEET SEEDLINGS[1]

W. F. BUCHHOLTZ[2]

(Accepted for publication January 24, 1938)

Seedling diseases constitute one of the chief limiting factors in obtaining maximum stands and yields of sugar beet, *Beta vulgaris* L. Fields with stands of less than 50 per cent occur in Iowa nearly every year. Sometimes entire fields are abandoned for beets and planted to other crops because of high seedling mortality. More frequently the damage is restricted to rather definite field areas.

The work here reported relates to the cause and control of these stand failures. When it appeared that *Pythium de baryanum* Hesse caused at least 95 per cent of the damage, the problem became a study of the biology of this organism in relation to its effect on the host. Such a study has involved, principally, the effect of temperature on the growth and pathogenicity of *P. de baryanum*, its response to pH, its relation to soil acidity and response to liming, and possibilities of control by seed treatment.

PERTINENT LITERATURE

Hellriegel (13) first suggested the parasitic nature of seedling troubles of sugar beets grown in field soil. He came to this conclusion after taking into account various nutritional and other causes, when he found that soaking seed in a weak solution of carbolic acid reduced the amount of disease. He attributed this effect to the disinfection of the seed ball. Frank (9) and Krüger (14) pointed out the importance of *Phoma betae* (Oud.) Fr. as a cause of sugar-beet-seedling disease, but Krüger suggested that the seedling trouble known as "Wurzelbrand" was probably a combination of several diseases involving several pathogens.

So many different causes had been ascribed to this trouble that Peters (19) wrote as follows concerning the matter:

[1] Journal Paper No. J-526 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project 432. Taken from a part of a thesis submitted to the graduate faculty of Iowa State College in partial fulfillment of the requirements for the degree, Doctor of Philosophy.

[2] These studies were conducted in the plant pathology laboratory of the Iowa Agricultural Experiment Station. The author wishes to express his appreciation to Dr. I. E. Melhus, who proposed the problem, offered many suggestions, and read the manuscript, and to Dr. C. S. Reddy, who also made suggestions during the course of experimentation.

Die Anzahl der angeblichen Wurzelbranderreger ist später noch um einige Pilze und Bakterien vermehrt worden, so dass noch kürzlich als Ursache des Wurzelbrandes nebeneinander Verhältnisse anorganischer Natur, Frass niederer Tiere und Parasitismus verschiedener Pilze und Bakterien angegeben wurden. Unter diesen Umständen schien es geboten die Frage in umfangreicherer Weise, als es bisher geschehen war, auf experimentellum Wege zu untersuchen und die verschiedenen Angaben, die im speziellen Teil eingehender gewürdigt werden sollen, einer experimentellen Nachprüfung zu unterziehen.

Peters (18) made his first report in 1906 and in his 1911 article (19) he thoroughly described and differentiated the symptoms brought about by *Pythium de baryanum* and *Phoma betae*. He pointed out very clearly that the former is soil-borne and acts much more quickly than *Phoma betae*, which he found to be seed-borne. Very few cultures of *Rhizoctonia* appeared in his isolations from diseased field-grown sugar beet seedlings, and he experienced difficulty in producing damping off by inoculation with *Rhizoctonia*. According to his observations, *Aphanomyces levis* de Bary, also soil-borne, invaded the young seedlings in a manner similar to that of *Pythium de baryanum*, but acted more slowly and, therefore, produced less severe damage.

Edson (7), working at Madison, Wisconsin, on sugar beets grown in mid-western and western soils, essentially confirmed in 1915 the work of Peters (18, 19). He found *Phoma betae* in his seed stocks but apparently it was not a very aggressive parasite. *Pythium de baryanum* and a new fungus, *P. aphanidermatum* (Edson) Fitzp., were the most destructive parasites involved. Beet seedlings grown on soils from western Kansas and Colorado yielded cultures mainly of *Rhizoctonia*.

Coons and Stewart (5) found that *Phoma betae*, *Pythium de baryanum* and *Rhizoctonia* were the causal agents of sugar-beet-seedling diseases in Michigan and devoted considerable effort to the development of chemical seed treatments. Previously, Miss Rumbold (21) had reported favorable results from sugar-beet-seed disinfection with formaldehyde and steam. Peters (19), in his work, had soaked seed in water at 60° C. for 10 minutes on two successive days to control seed-borne *Phoma betae*. Coons and Stewart (5) reported that formaldehyde, furfural, nickel carbonate dust, Kalimite, Tillantin B and Seed-O-San were of little or no value in their experiments, but that Semesan, DuPont Semesan 13, Chlorophol, Uspulun and mercury bichloride had given marked stand improvement. Copper carbonate and copper sulphate-lime dusts were intermediate in control value.

Arrhenius (1), working in Sweden, found an association between soil acidity and seedling diseases of beets. He reported that analyses of soil from infested fields showed an acid reaction in every case. The most favorable reaction for the development of beets and the control of their seedling diseases appeared to be pH 7.2 to 7.6, which, he stated, could be secured by applying lime in suitable quantities. Cultural studies with *Pythium de baryanum*, which he considered to be the most frequent cause, showed that the growth of the fungus was almost entirely inhibited at pH 7.5. He also pointed out that more serious damage occurred in later-planted fields, espe-

cially on areas of acid soil. Molz (17) in Germany, Gäumann (11) in Switzerland, Marchal (16) in Belgium, Gallagher (10) in Ireland, Hartwell and Damon (12) at Rhode Island, and others have likewise noted failures of sugar beets on acid soils and improvements in stand and yield from the addition of lime.

THE NATURE AND CAUSAL AGENT OF STAND FAILURES IN NORTHERN IOWA

The severity of losses from stand troubles in northern Iowa is variable. In nearly all fields there is some seedling mortality, but the practice of sowing an excess of seed and selectively thinning usually establishes a stand of 50 to 80 per cent. Even such practices, however, fail to establish stands when damping off is severe. Such a condition is shown in figure 1. In this area less than 20 per cent of a stand was marketable, and the yield was less than a ton per acre. Stands as poor as this seem limited to certain fields, and the most severe damage has been on localized well-defined areas within a field.

FIG. 1. A spot in a sugar-beet field near Kanawha, Iowa, in which the stand was destroyed by damping off in 1932.

Seedlings grown in the greenhouse and in the field in soil taken from such areas exhibit symptoms similar to those shown in figures 2 and 3. In 1932 the examination of many such plants was undertaken. Isolations from a large number of diseased seedlings with 3 or 4 leaves (Fig. 3) resulted in a very low yield of a variety of fungi. It soon became evident, however, that such seedlings probably were not at the stage of primary infection. To prevent excessive contamination of isolation plates by soil bacteria that

FIG. 2. Sugar-beet seedlings grown in the greenhouse on soil from the area shown in figure 1. The soil in the pot at the right was steamed.

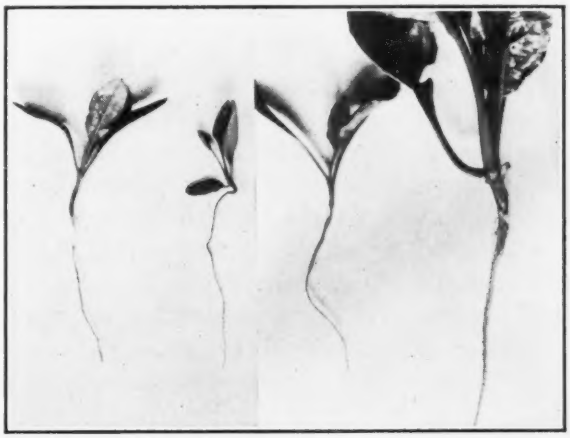

FIG. 3. Growing sugar-beet seedlings of two different ages: the two on the outside healthy, the two toward the center with symptoms of cortical invasion of the hypocotyl by *Pythium*.

had invaded these advanced lesions, it was necessary to surface disinfect so heavily that there was also a possibility of killing the pathogen if it were still present. In subsequent isolation trials, water-soaked but not collapsed portions of recently invaded younger seedlings were placed on potato-dextrose agar plates after very little, preferably no surface disinfection with mercuric chloride. Such a procedure seldom yielded any fungus cultures other than of *Pythium*. The same method yielded cultures of *Phoma betae* from infected seedlings grown from nontreated seed on steamed soil in the greenhouse and, on rare occasions, from field specimens that were not entirely killed by *Pythium* or that were grown under conditions (such as low temperature) unfavorable for *Pythium*.

Cultures of *Rhizoctonia* have rarely been recovered from field-grown seedling beets. Such cultures, introduced into steamed soil in the green-

house, have induced necrotic lesions on seedlings. It seems improbable that numerous isolation tests would have yielded so few *Rhizoctonia* cultures if this pathogen were responsible for considerable damage in the field.

Prevalence and Development of Phoma betae, Pythium and Rhizoctonia

Numerous isolations from diseased seedlings grown in the field and in the greenhouse resulted in the recovery of *Pythium* from a large majority of both field and greenhouse specimens. Subsequent inoculations showed that such cultures of *Pythium* were highly pathogenic. Cultures of *Rhizoctonia,* while also pathogenic, were not so virulent, and were seldom recovered from either field- or greenhouse-grown seedlings. *Phoma betae* proved to be even less pathogenic than *Rhizoctonia* and was recovered infrequently, except from seedlings grown from infected seed on steamed soil in the greenhouse.

To observe and compare the relative pathogenicity of *Pythium, Rhizoctonia* and *Phoma betae* under controlled conditions, cultures of *Pythium* and *Rhizoctonia* were introduced into steamed soil in the vicinity of germinating or emerging sugar-beet seedlings grown from *Phoma betae*-infected seed at the same time that sugar beets were growing from the same lot of seed on several field soils and noninfested steamed soil, as follows:

Two rows of 10 sugar-beet seed balls were planted in each of 32 pots of steamed Clarion loam with a 2-ton lime requirement. In 8 of the pots, 1 row of seed was treated before planting with ethyl mercuric phosphate dust (5 per cent, $\frac{1}{4}$ g. per 100 g. of seed). In 8 others, 2 small portions of a pathogenic *Rhizoctonia* culture were placed in contact with the seeds in 1 row at the time of planting. In 8 others the *Rhizoctonia* culture was placed in contact with 1 row of emerging seedlings, as were 2 similar portions of a pathogenic *Pythium de baryanum* culture, in the remaining 8 rows. In each pot, 1 row was left without either treatment or inoculation to serve as a check.

In like manner, in each of 8 pots of nonsteamed Clarion loam from Kanawha, 8 pots of nonsteamed Clarion loam from Ames, and 16 pots of nonsteamed, neutral Webster silty clay loam from Ames, were planted 1 row of 5 treated and 1 row of 5 nontreated seed balls. Fewer seed balls were planted in the nonsteamed field soil to avoid excessive spread of the pathogen from one group of seedlings to another, so that the disease counts would more nearly estimate the actual soil infestation. The counts were then doubled to make them comparable in value to those taken from the steamed soil.

Two readings were taken, the first on March 11 as soon as seedlings in the steamed Pythium-infested soil began to die; the second on March 18 and 19, when the seedlings were dug (Table 1). At the first reading, the nontreated rows on the nonsteamed Clarion loam had the poorest stand of any in the experiment. Rhizoctonia infestation at planting time also resulted

in a poor stand, but infestation at emergence had not produced any disease, whereas about 20 per cent infection had resulted from Pythium infestation. Almost no symptoms of *Phoma betae* infection were visible on seedlings grown from nontreated seed in steamed soil. At the second reading, there were only occasional healthy seedlings from nontreated seed on nonsteamed Clarion loam and in the Pythium-infested rows on steamed soil. By this time Rhizoctonia infestation at emergence had produced considerable infection, and the earlier infestation had spread across to the check row. Considerable *Phoma betae* infection also had developed on seedlings from nontreated seed in steamed soil. This was largely controlled by the ethyl mercuric phosphate dust, as was the Pythium infection on nonsteamed Clarion loam. On nonsteamed Webster silty clay loam, there was no more disease than on steamed soil.

Results of this experiment confirmed the previous field and greenhouse observations that were supported by isolations and subsequent infestation and inoculation trials similar to parts of this experiment.

Symptoms of Phoma betae infection. In this experiment, *Phoma betae* caused evident injury only in the steamed soil and in the Webster silty clay loam, which apparently was only lightly Pythium-infested. In both cases it was controlled by seed treatment. *Phoma betae* has been commonly found in seed lots and observed to infect sugar-beet seedlings grown in steamed soil. It eventually will produce an extensive cortical lesion, but, for at least 10 days after emergence of the seedling under greenhouse conditions, there was visible only a constantly darkening tan to brown area at the base of the hypocotyl, which developed progressively from the point of contact with the seed ball. Sometimes a brown necrotic streak extended up and down from what appeared to be the point of infection. The mycelium is at least temporarily restricted to the intercellular spaces and the infected hypocotyl is more brittle than is normally the case.

Gradually, the invaded cortex turns black and, in many instances, collapses. The plants may fall over, but, frequently, under favorable soil and other conditions, continue to live and grow. Apparently, the invading hyphae seldom penetrate to the vascular cylinder and, if growth conditions are sufficiently favorable, an infected hypocotyl may continue to thicken despite the condition. Eventually, the dead cortical tissue disintegrates and is replaced by secondary tissue. In the greenhouse this process ordinarily took 2 or even 3 weeks after the emergence of the seedling.

Symptoms of Pythium infection. If *Pythium* is abundant and active in the soil, it may destroy 95 per cent of the seedlings before seed-borne *Phoma betae* makes any progress (Table 1). The pure-culture infestation with *Pythium de baryanum* made on March 9 brought about the infection of more seedlings in 2 days than were found to be infected with seed-borne *Phoma betae* 10 days after emergence. Many of the seedlings in Clarion loam did not emerge. Some were invaded in the seed ball. This has not been the case in the field, where many have been invaded shortly after appearing outside

TABLE 1.—*Average number of healthy and diseased sugar-beet seedlings from greenhouse-planted rows of Phoma betae-infected seed in steamed soil, in soil naturally infested with Pythium and soil artificially infested with Pythium de baryanum and Rhizoctonia*

Soil	Average number of healthy and diseased seedlings											
	March 11						March 18 and 19[a]					
	Row of treated seed[b]		Check		Row in artificially infested soil		Row of treated seed		Check		Row in artificially infested soil	
	H	D	H	D	H	D	H	D	H	D	H	D
Neutral Webster silty clay loam, not steamed	27	0	26	0.5			28	0	23	3		
Acid Clarion loam, not steamed	26	0	6	3			24	4	2	7		
Acid Clarion loam, steamed	27	0	25	0			26	1	21	5		
Acid Clarion loam, steamed:												
Rhizoctonia infestation, March 2			25	0	12	1			13[c]	13	8	6
Rhizoctonia infestation, March 9			25	0	26	0			22	5	11	16
Pythium infestation, March 9			24	0	19	5			21	4	4	20

[a] Seedlings dug up at this time.
[b] Seed treated with ethyl mercuric phosphate dust, 5 per cent, ¼ g. per 100 g. of seed.
[c] Infestation had spread across pot to noninfested row.

the seed coat or shortly before emerging from the soil. In these cases, and in many instances of infection of emerged seedlings, the invasion extends into the vascular, as well as the cortical, tissue so that wilting ensues and the seedlings die quickly. Young, emerging seedlings, when thus infected, rapidly become desiccated, especially in the field.

The initial Pythium infection appears as a slightly darkened, glistening, water-soaked spot, usually occurring about ½–2 inches below the surface of the soil, depending on moisture level and depth of planting. The region of the hypocotyl, just at and above the base of the collet, seems to be the most susceptible part of the young seedling. A result of invasion of the vascular cylinder is a blackening of that portion of the axis, even beyond the extent of the cortical lesion. Young seedlings so invaded die quickly. When no vascular invasion occurs, the seedlings may continue to live and grow (Fig. 3). As it persists, the growing cortical lesion first turns to a chestnut brown, then collapses, and turns black. Sometimes it extends to the base of the cotyledons. The rapidity of such extension as compared to the general vigor and rate of growth of the seedling apparently determines the ultimate fate of the plant.

Occasionally, the central axis may be severed and the plant yet remain alive. Under conditions of sufficient moisture, roots may develop from the lowermost portion of the living axis just above the severed region. Such individuals apparently become what might well be called "turnip" beets. They are undersize, short, round, and usually develop several to many branch roots. Commercially, they are worthless, rarely attaining a weight of ¼ pound.

Symptoms of Rhizoctonia Infection. Rhizoctonia injury of seedlings, under northern Iowa conditions, is difficult to evaluate or describe. It was rarely obtained in isolations from field-grown diseased seedlings, especially very young ones. Some cultures have proved pathogenic to small seedlings, while others have brought about but little injury. Rhizoctonia lesions in the above experiment differed from those induced by *Pythium* in that they became a dark brown or chocolate, shortly after infection, and were less water-soaked.

It is not particularly difficult to recognize the above described distinctions between the types of symptoms produced by these 3 different pathogens when the mass effects of each are available for observation, but in the field it is not possible to determine the particular organism involved in the individual cases without resort to isolations.

Under northern Iowa conditions, at least 95 per cent of the damage to seedling sugar beets appears to be due to *Pythium*. This statement is supported by the following findings: (1) Isolations from young seedlings grown in the field or on field soil in the greenhouse give almost 100 per cent *Pythium* cultures unless seedlings were grown in cold or lightly Pythium-infested soil; (2) *Pythium*, which occurs naturally in field soil, and that introduced into steamed soil is much more rapid and destructive in its action

than either *Rhizoctonia* or seed-borne *Phoma betae;* (3) symptoms that occur on seedlings known to be infected with *Pythium* in the greenhouse are similar to the symptoms observed on young seedlings in the field.

Identity of the Pythium Isolates from Sugar Beets

Although not identical, a majority of the *Pythium* isolates, taken from diseased sugar beet seedlings grown in northern Iowa were enough alike to be considered the same species. Their young mycelium branched freely, was nonseptate, relatively hyaline, and from 1.5 to 3.5 μ in diameter. Older mycelium was larger and septate, and seemed to be devoid of protoplasm. When a portion of a culture was transferred to sterile distilled water, spherical or slightly ovate conidia, 10 to 35 μ in diameter, were found terminally on branches of the newly-formed mycelium. Similarly formed, intercalary conidia were of the same size and spherical to ellipsoid in shape. If chilled at 0°–10° C. for 1–6 hours and then transferred to carrot decoction or malt extract, the conidia germinated by a germ tube in 15 to 20 minutes to 4 hours at room temperature, the time being fairly characteristic of a given culture. Zoospore production was not observed.

Occasionally oospores were produced simultaneously with, or soon after, the formation of conidia from cultures in distilled water. They formed more readily in the tissues of an infected host plant that had been left floating in distilled water 4–7 days. The oospores averaged 13.6–17 μ in diameter; and some contained a central globule. The oospore walls were smooth and varied in thickness from 1.5–2 μ. The mature oospores were free in the oogonium. The oogonia averaged from 16.6–22.1 μ in diameter; their thin walls were membranous and smooth. Not more than 2 androgynous antheridia were observed with a single oogonium.

With the exception of the failure of zoospore formation, which seems also to have been the experience of Atkinson (2), Ward (22), and Butler (3, pp. 86–91), the characteristics of the *Pythium* isolates taken from diseased sugar-beet seedlings grown in northern Iowa would classify them in the species, *Pythium de baryanum* Hesse; and it seems probable that stand failures, in northern Iowa, are caused by the same organism, encountered first by Peters (19), in Germany, and later by Edson (7) and Coons and Stewart (5) in the United States.

TEMPERATURE RELATIONS OF PYTHIUM DE BARYANUM

It is a common experience among beet growers in northern Iowa that early plantings produce better stands and higher yields, so much so that the observing and successful growers rarely delay their plantings later than May 10; the most consistently successful growers rarely fail to plant in April. These observations suggested the possible relation of soil temperature to the abundance and pathogenicity of *Pythium de baryanum* in the soil.

Temperature and Growth Rate in Pure Culture

A pathogenic culture of *Pythium de baryanum* was subjected to each of 9 different temperatures in the following manner: In the center of each of 54 potato-dextrose agar plates was planted a small piece of agar with rapidly growing mycelium. These plantings were allowed to stand at room temperature for 8 hours to become established and commence to grow. At this time the colonies were 1 to 2 cm. in diameter. Their margins were plotted on the glass at 4 points. Subsequently, the cultures, 6 at each temperature, were incubated at 5, 9, 12, 15, 20, 25, 30, 35, and 40 degrees C. for 12 hours. Two measurements of increase in diameter of each colony were averaged to yield the data shown in figure 4. There appears to be a rather distinct upward break in the curve between 12° and 15° C. Maximum growth was obtained at 30° C., with a very sharp drop at 35° C.

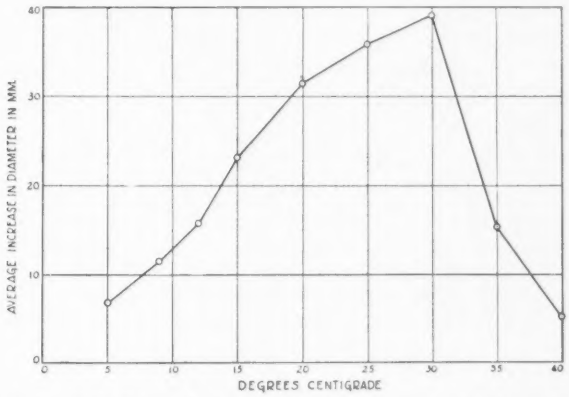

FIG. 4. Growth of *Pythium de baryanum* in 12 hours at various temperatures.

It seemed that neither the maximum nor the minimum temperature had been included in the series. Furthermore, growth was so rapid that some increase in diameter probably took place in plates at the low and high temperatures before the entire mass of 6 plates had been adjusted to those temperatures. Accordingly, the entire experiment was repeated in the same manner, except that after growth had started the plates were allowed to remain in the respective incubators approximately 2 hours before the margins were first plotted. With this procedure no growth occurred on any of the plates at 1° and 5° C., and a measurable growth on only one of the plates at 40° C. Otherwise, the results were similar to those described in the previous experiment.

Temperature and Pathogenicity in Field Soil and in Pure Cultures

A series of experiments was designed to determine the relative amount of emergence and damping off of beet seedlings grown in naturally infested field soil and artificially infested steamed soil at various temperatures. In the first of these, 36 pots of naturally infested field soil, planted with 5 seed

balls each, were incubated, 4 at each of 9 temperatures, 5, 9, 12, 15, 20, 25, 30, 35, and 40 degrees C. All were adequately watered, but no attempt was made to maintain a constant moisture content. No light was available to the seedlings after emergence. Emerged and diseased seedlings were counted daily until the plants at the higher temperatures were all dead or very etiolated and abnormal; then they were discarded and subsequent counts were taken every other day on those at the lower temperatures. As soon as an individual seedling showed infection it was removed to avoid spread of the pathogen from it to others near-by. *Pythium* was recovered from diseased seedlings at all temperatures (Table 2). The numbers, emerged and diseased, are shown in figure 5.

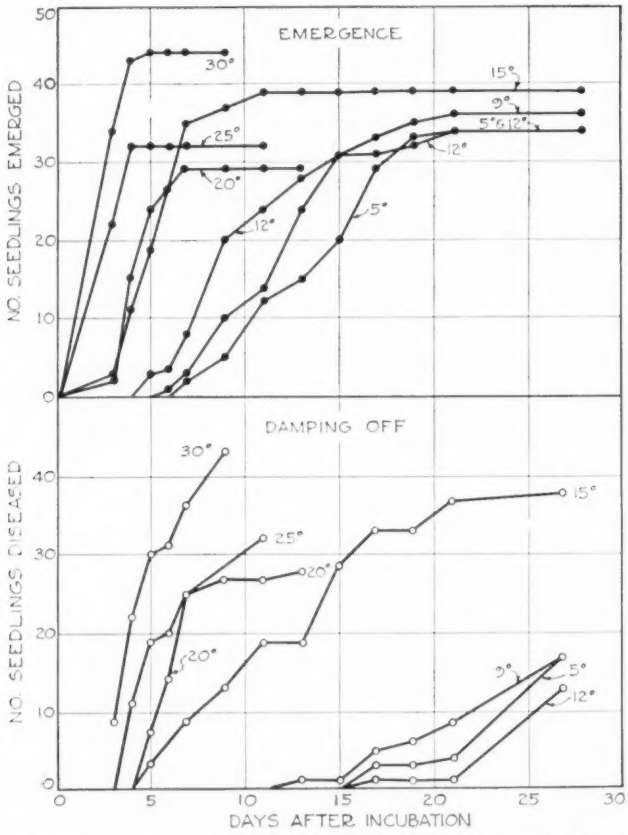

FIG. 5. Rate of emergence and damping off of sugar-beet seedlings grown on naturally infested field soil at various temperatures (C).

It is evident that at 5°, 9°, and 12° C., while the rate of emergence and damping off were both slow, the latter was enough slower that, when the experiment was discontinued, some plants were still healthy. At temperatures of 20°, 25°, and 30° C., infection closely followed rapid germination, and at the time of discontinuation only one healthy plant remained at 30° C. The data taken at 35° and 40° C. were not included in figure 5 because, at

these temperatures, the seedlings quickly became extremely etiolated. Nevertheless, the pots at these temperatures did yield less damping off than did those at 20°, 25°, and 30° C.

No explanation can be offered for the apparently slow rate of damping off at 12° C. The 15° C. incubator fluctuated upward several times, so the germination rate and damping off represented for that temperature may be a little high. Even so, the incidence of disease at 12° C. is below 9° and 5° C.

TABLE 2.—*Recoveries of Pythium from diseased seedlings grown in field soil at different temperatures*

Temperature °C.	No. seedlings plated	No. times Pythium recovered
5	17	4
9	17	15
12	13	10
15	20	14
20	13	11
25	12	12
30	12	12
35	12	12
40	8	5
Total	132	95

In a second experiment, a duplicate of the first, except that 35° and 40° C. incubations were omitted and a different collection of infested field soil was used, emergence and damping off at 12° C. were intermediate between 9° and 15° C. In the latter experiment, however, fewer seedlings emerged at lower temperatures, which was not the case in the experiment shown in figure 5. The lag in damping off, compared to emergence, was again evident at the lower temperatures.

In a third experiment, 8 pots of a third collection of naturally infested field soil and 8 pots of steamed, artificially infested soil, each planted with 5 graded seed balls, were incubated at 5, 10, 15, 20, and 30 degrees C. The emerged and diseased seedlings at each temperature were counted when it was thought no more emergence would take place. All seeds were dug up and unemerged seedlings also were classified as healthy or diseased. The comparisons appear in figure 6.

It will be noted that the field soil used in this experiment produced less damping off than occurred in the previous experiments. Nevertheless, the total amounts and percentages of damping off on field soil were greater at the higher temperatures. The results on steamed soil artificially infested with *Pythium de baryanum* are especially noteworthy. Infestation was accomplished by covering the seed, which had previously been planted on the surface of the steamed soil in a pot, with a $\frac{1}{4}$–$\frac{1}{2}$ ich layer of steamed soil that had been inoculated in a flask 2 weeks earlier with an agar culture of *Pythium de baryanum*. The infestation apparently was so heavy that at 15°, 20°, and 30° C. very few of the seedlings emerged, and all of those that

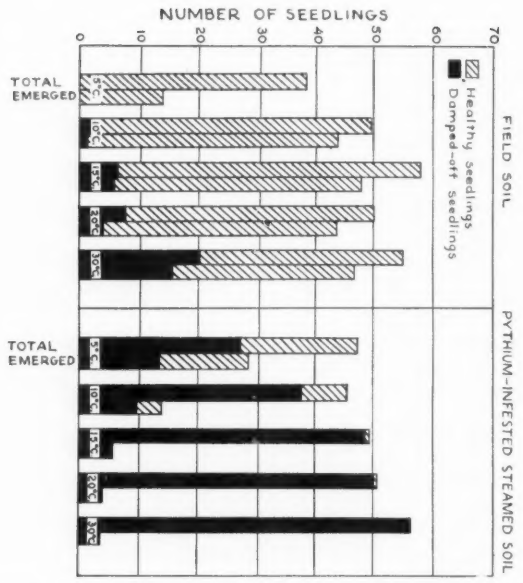

FIG. 6. Numbers healthy and diseased of total (nonemerged plus emerged) and emerged sugar-beet seedlings grown in field soil and Pythium-infested steamed soil at various temperatures.

did became infected. At 5° C. there was about 50 per cent emergence, and of the resultant seedlings nearly one-half were healthy. The results at 10° C. were intermediate. In both field soil and infested steamed soil there were some healthy seedlings that had not emerged, especially at 5° and 10° C.

In all three of the experiments just described, temperatures of 15° C. or below were more unfavorable to damping off than to germination and emergence. This evidence supports the experience of growers with early plantings and has been further substantiated in results from field experiments described elsewhere in this paper.

SOIL FACTORS ASSOCIATED WITH DAMPING OFF IN THE FIELD

The severe damage in rather well-defined areas (Fig. 1) is one of the striking features of damping off of sugar beets in the field. On part of a field the stand may be satisfactory, while only a few rods away there may be a complete stand failure. Data taken in 1932 from 2 such adjacent areas are presented in table 3.

Around Kanawha such heavily infested areas usually consist of Clarion loam or Webster loam. They are slightly more elevated than the lightly infested areas, which fall on the lower edge of the Webster silty clay loam and on the Lamoure silty clay loam. The heavily infested soil appears to be somewhat lower in organic matter and not so loose and friable, but to all appearances it produces nearly as good crops of corn and small grain as does the lightly infested soil.

TABLE 3.—*Sugar-beet stands harvested in 1932 from adjacent areas in the same field at Kanawha, Iowa.*

Heavily infested soil area			Lightly infested soil area		
Stand	Marketable beets	Perfect stand marketable	Stand	Marketable beets	Perfect stand marketable
Per cent[a]	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
50	32	16	102	98	100
44	45	20	64	100	64
54	7	4	58	96	56
56	43	24	66	100	66
36	67	24	106	94	100
Ave. 48	38.8	17.6	79.2	97.6	77.2

[a] One beet per linear foot of row was considered a 100 per cent drilled stand. Beets with a root diameter of 1 inch or more were classed as marketable.

Soil Moisture in Areas of Good and Poor Stands

It was thought that the high percentage of damping off in these heavily infested areas might be the indirect result of excessive soil moisture, even though these areas are on gentle slopes or at the tops of knolls. In the fall of 1932, soil moisture determinations were made at various depths to 30 inches in 4 areas, 2 with good and 2 with poor stands. Duplicate borings were made with a soil auger and the moisture content was determined by the standard procedure, on an oven-dry basis. The moisture content of the surface six inches was slightly higher where stands were good than where thin, although on the basis of percentage of saturation the difference was only 1.35 per cent. There was very little difference in subsoil moisture.

In 1934, surface-soil moisture determinations were made twice a week on 2 locations in an experimental plot, one where the stand was good, the other where it was poor. The average moisture content of the latter (I) was 21.89 per cent, for the former (II), 23.13 per cent (Table 4). The moisture-holding capacity was 49.55 per cent in Area I and 55.55 per cent in Area II, so that on the basis of percentage saturation, Area I was more moist on the average than Area II. For a short period following rains, the soil appears noticeably more moist in Area I than in Area II. Sugar-beet seeds germinated more quickly in Area I. A comparison of the data obtained by averaging all the determinations in which both areas had a moisture content of 20 per cent of dry weight or more and all determinations in which one or both of the areas was below 20 per cent shows that at the higher moisture contents, the difference between the averages for the two areas is only 0.94 per cent (Table 4). At the lower contents, the difference is 1.63 per cent. On the basis of percentage saturation, Area I is 4.74 per cent more moist at the higher moisture contents, but actually 0.26 per cent less moist at the lower moisture contents. It may be that during periods of high moisture following considerable precipitation there is more free water in the soil of

Area I, even though the actual moisture content may be no greater or even slightly less. Even so, it is not probable that a difference even as large as 4.74 per cent during periods of high moisture would mean the difference between a good stand and a complete failure, as has repeatedly been the case in these adjacent areas.

Relation of Damping Off to Soil Acidity

In the fall of 1932 in 3 different fields at Kanawha that had poor stands of sugar beets, the soil in these areas had a lime requirement of ½ ton to 3 tons per acre. On adjacent neutral areas in the same field, stands were good. In one case, when sugar beets were planted on such soils in the greenhouse, only 28 healthy plants remained in 16 rows on acid soil, as against 372 on the neutral soil.

TABLE 4.—*Average soil moisture contents of an area with poor stand (I) and an area with good stand (II)*

Moisture content	Area with poor stand (I)		Area with good stand (II)		Difference (I)–(II)	
	Dry wt.	Saturation	Dry wt.	Saturation	Dry wt.	Saturation
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Average of 24 high-moisture contents	29.52	59.57	30.46	54.83	– 0.94	4.74
Average of 19 low-moisture contents	12.25	24.72	13.88	24.98	– 1.63	– 0.26
Average of 43 moisture contents	21.89	44.17	23.13	41.63	– 1.24	2.54

During the 1933 season a field survey was made in Hancock, Wright, and Cerro Gordo counties, including especially those fields that showed good and poor stands of beets in adjacent areas. One composite soil sample together with 4 counts of thinned stand were taken in each area of poor and good stand. Lime requirement and pH determinations were made in duplicate for each soil sample. Lime requirement determinations were made with the Emerson (8) soil-test solution. When this solution is mixed with an acid field soil, a red color is produced, and the lime requirement of the soil is estimated by comparison with a standard color chart. Determinations of pH were made with Lamotte indicators and standards, after the method of Clark and Lubs (4). A measured amount of supernatant solution from a settled mixture of soil and distilled water was decanted off, a standard amount of indicator added, and color comparisons made. Occasionally a sample was slightly cloudy, but by the use of similar checks in the comparator block it was possible to estimate to the nearest one-tenth of a pH value. The indicators most commonly used were brom thymol blue (pH 6.0–7.6), chlorphenol red (pH 5.2–6.8), and, occasionally, phenol red (pH 6.8–8.4).

TABLE 5.—*Percentage stands of sugar beets on northern Iowa soils with and without lime requirements in 1933*

No. soils	Lime requirement, tons per acre		Soil pH range	Percentage stand	
	Average	Range		Average	Range
21	1.89		(Not determined	47.04	
17	0.00		for 10 samples)	76.26	
15 (See	2.06	0.5–3.0	5.95–6.5	41.4	10.5– 75.0
13 text)	0.00		6.4 –7.6	79.15	38.5–107.5
14	2.04	0.5–3.0	5.95–6.5	39.0	10.5– 64.2
12	0.00		6.4 –7.6	82.54	60.0–107.5

Correlations calculated from lime requirement, soil pH and percentage stand data

Correlated variables	Correlation coefficient	Significant values
Lime requirement and percentage stand	– 0.556	0.325 0.418[a]
Soil pH and percentage stand	0.745	0.374 0.478[a]
Lime requirement and soil pH	– 0.721	0.349 0.449[a]

[a] Highly significant.

Soil pH values, average lime requirements and stand counts, and correlation coefficients are presented in table 5. They show that in the fields visited there was a highly significant correlation between soil acidity and

TABLE 6.—*Lime requirement, soil pH determinations, and thinned stand counts taken in 1934 survey of sugar beet fields in northern Iowa*

Lime requirement, tons per acre	Soil pH	Percentage stand
	Soils with pH 6.5 or less	
2	6.0	55
1	6.45	68
2	6.0	68
0.5	6.3	73
1	6.35	33
2	6.05	24
1.5	6.1	62
1	6.5	50
1.5	6.25	36
2	6.2	38
Average 1.45		50.7
	Soils with pH 6.6 or more	
0	6.65	79
0	7.25	100
0	7.1	92
0.5	6.6	73
0	6.7	67
0	6.85	63
0	7.0	69
0	6.95	95
0	6.9	85
Average 0.055		80.3

poor stands. The lime requirement test divided these soils approximately into 2 groups, those showing a lime requirement and a pH of 6.5 or less, and those without a lime requirement and a pH of 6.4 or more. With one exception in each group, the soils without a lime requirement had a stand of 60 per cent or more and those with a lime requirement had a stand of 64.2 per cent or less.

In 1934 the same procedure (Table 6) was carried out. Not so many fields were visited as in 1933. From the table it is again evident that the more acid soils had poorer stands.

Effect of pH on the Growth Rate of Pythium de baryanum

When the possibility of a correlation between soil acidity and poor stands caused by *Pythium de baryanum* presented itself, it was first thought that the pathogen probably was distinctly favored by an acid medium. Accordingly, several lots of potato-dextrose agar were adjusted with lactic acid and sodium hydroxide to different degrees of acidity and alkalinity ranging from pH 4.0 to pH 8.1. Four plates were poured for each pH value and transfers were made in duplicate for each pH value of two pathogenic cultures of *Pythium de baryanum*. Twelve-hour growth increments were recorded by measuring increase in radius of each plate culture in four places. Results appear in figure 7.

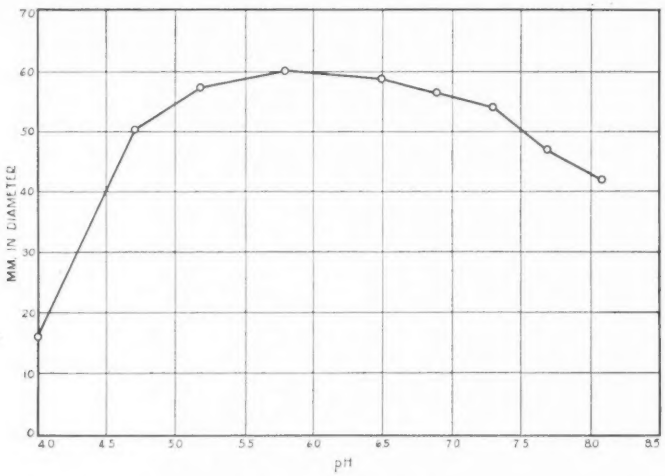

FIG. 7. Growth of *Pythium de baryanum* in 12 hours on potato-dextrose agar adjusted to pH 4.0–8.1.

The most growth occurred at pH 5.8, which would indicate a tolerance for a slight degree of acidity. However, growth at pH 4.7, 5.2, 5.8, 6.5, 6.9 and 7.3 was very nearly the same (between 50 and 60 mm.), and growth at pH 8.1 was greater than at pH 4.0. If these results are indicative of the growth of *Pythium de baryanum* in the soil, one would expect soils with pH values from 4.7 to 7.3 to contain approximately equal amounts of *Pythium*.

The evidence already presented points to the fact that there was more damping off of sugar beets in acid soils.

FIELD RESULTS WITH LIMING, SEED TREATMENT, AND DATE OF PLANTING

The preliminary field observations and greenhouse and laboratory data, thus far presented, have emphasized the relation of soil acidity and soil temperature to the abundance and pathogenicity of *Pythium de baryanum*, the chief cause of stand failure in the field, and have suggested the possibility of control by seed treatment. Field experiments, therefore, were designed to test the value of liming acid and neutral soils, early planting to take advantage of low soil temperature early in the spring, and seed treatment. On Dec. 23, 1932, sugar factory lime cake was applied at the rate of 1.25 tons per acre, air-dry weight, one each of 4 strips of soil that alternated with 4 nonlimed strips. The entire plot extended across an acid and a neutral soil area. On April 24, 1933, 1 ton per acre was added to the previously limed strips and the first planting was made on April 29 on 6 of the strips, 3 limed and 3 not.

This plot had been divided into 14 smaller plots, each of which crossed all 6 strips. Six plots were on acid soil, 6 on neutral soil, and 2 in an intermediate position. The plan was to plant on each plot 1 replication of a

TABLE 7.—*Seed treatments applied in the field experiment of 1933*[a]

Number	Toxic agent	Adhesive properties
1	Ethyl mercuric chloride, 2 per cent	Very good
2	Ethyl, mercuric phosphate, 5 per cent, ¼ g. per 100 g. of seed	Excellent
3	Ethyl mercuric phosphate, 5 per cent, 1 part, plus 3 parts hydrated lime	Good
4	Ethyl mercuric chloride, 2 per cent, 50 g. Mercuric chloride 2 g. Hydrated lime 48 g.	Intermediate
5	Ethyl mercuric phosphate, 5 per cent, 20 g. Mercuric chloride 2 g. Hydrated lime 78 g.	Intermediate
6	Ethyl mercuric chloride 10 g. Hydrated lime 90 g.	Intermediate
7	Hydrated lime dust (dry seed balls rolled in excess of dust)	Fair
8	Hydrated lime wet roll (wet seed balls rolled in excess of hydrated lime and allowed to dry)	
9	Diphenyl mercury, 2 per cent	Fair
10	Ethyl mercuric chloride, 2 per cent, 33⅓ g. Mercury furfuramide, 5 per cent, 66⅔ g.	Intermediate
11	Formaldehyde dust	Poor
12	Ethyl mercuric phosphate 25 g. Colloidal phosphate 75 g.	Good

[a] All dust treatments applied at rate of 1 g. per 100 g. seed balls, except treatments 2 and 7. Results in table 9.

sugar-beet seed-treatment experiment, each replication to consist of 4 check rows and 1 row of each of 12 seed treatments. Graded seed balls were used, 1 per hill, in hills 1 foot apart. The rows were 2 feet apart. Twenty-one hills were marked off in the center of each 28-foot strip for counting. Emergence and survival counts were to be taken at intervals, and stand counts and yields at harvest time. The seed treatments used are enumerated in table 7.

Five replicate plots were planted on April 29 and May 1, 2 on acid soil, 2 on neutral soil, and 1 in an intermediate position. A second similar planting was made on May 16. Emergence data had just been collected on the first planting when, on May 23, both plantings were destroyed by wind. From the last planting, made on May 26, emergence and a set of survival data were taken before it became evident that there would not be a measurable yield. The experiment was then plowed up. Soil temperature and rainfall data for the time of germination and emergence for the early (April 29, May 1) and the late (May 26) plantings appear in table 8.

TABLE 8.—*Soil temperatures and rainfall during germination and emergence period for early (April 29, May 1) and late (May 26) plantings in 1933*[a]

	Date planted	Date of emergence reading	Rainfall, inches	Soil temperature, °C.				
				a. m.		p. m.		Ave.
				Ave.	Range	Ave.	Range	
Early planting	Apr. 29–May 1	May 18	2.25, during 9 days	8.5	5.6–13.6	11.3	7.5–14.7	9.89
Late planting	May 26	June 7	0.8+ during 3 days	19.0	12.5–24.0	26.2	16.5–33.0	22.2

[a] Refer to table 9.

Field Results, 1933. While it was evident that no satisfactory control had been devised, some of the data collected indicated possibilities. Comparative total emergence from the early and late plantings is shown in table 9.

It is observed that more seedlings emerged from the early planting than from the late planting, especially on acid soil; the late planted check yielded 50 per cent more emerged seedlings on neutral soil than on acid soil; the early planted check yielded nearly as many emerged seedlings on acid soil as on neutral soil; in the late planting the best seed treatment (No. 1) increased emergence 37 per cent on acid soil and only 7 per cent on neutral soil; in the early planting the best seed treatment increased emergence 52 per cent on acid soil and 34 per cent on neutral soil; and liming did not affect emergence in the early plantings, but did bring about an increase of 12.5 per cent on acid soil and a 4.6 per cent increase on neutral soil in the late planting.

Table 10 contains emergence data from a slightly different combination of the late planted plots, with one set of counts seven days after emergence.

TABLE 9.—*Number of sugar-beet seedlings emerged on limed and nonlimed acid and neutral soil from early and late plantings of treated and nontreated seed, 1933*

Seed treatment[a]	Planting date[b]	Acid soil		Neutral soil		Totals	Rank of seed treatments
		Nonlimed	Limed	Nonlimed	Limed		
Check	Early (April 29–May 1)	192	188	190	200	770	
	Late (May 26)	124	160	206	219	710	
1—C_2H_5HgCl, 2%	Early	299	280	243	281	1103	1
	Late	187	201	226	230	844	1
2—$(C_2H_5Hg)_3PO_4$, 5%	Early	277	301	271	243	1092	2
	Late	187	190	223	213	813	4
3—$(C_2H_5Hg)_3PO_4$ + hydrated lime	Early	277	288	251	244	1060	4
	Late	186	206	193	250	835	2
4—$C_2H_5HgCl + HgCl_2$ + hydrated lime	Early	260	257	245	266	1028	6
	Late	169	198	189	204	760	8
5—$(C_2H_5Hg)_3PO_4 + HgCl_2$ + hydrated lime	Early	270	258	269	270	1067	3
	Late	177	213	194	207	791	6
6—C_2H_5HgCl + hydrated lime	Early	222	201	180	163	766	10[c]
	Late	144	184	183	201	712	11
7—Hydrated lime dust	Early	201	210	221	172	804	9
	Late	161	153	203	223	740	9
8—Hydrated lime wet roll	Early	199	180	181	184	744	12[c]
	Late	161	154	186	205	706	12[c]
9—Diphenyl mercury, 2%	Early	241	236	224	265	966	8
	Late	164	179	219	212	774	7
10—C_2H_5HgCl + mercury furfuramide	Early	253	263	236	232	984	7
	Late	154	204	233	238	829	3
11—Formaldehyde dust	Early	193	182	195	178	748	11[c]
	Late	149	170	211	204	734	10
12—$(C_2H_5Hg)_3PO_4$ + colloidal phosphate	Early	260	276	242	257	1035	5
	Late	174	203	217	202	796	5
Totals	Early	3144	3120	2948	2955		
	Late	2137	2415	2683	2808		

[a] Complete formula in table 7.
[b] Refer to table 8 for soil temperature and rainfall data.
[c] Less than from nontreated check.

The difference between acid and neutral soil is much more noticeable in the later count than in the one taken at the time of emergence. It can be seen that liming produced a slight increase in the number of emerged seedlings on acid soil but not on neutral soil. More seedlings survived on the limed plots, especially on acid soil. Soon after the first survival counts were made, the stand was so uniformly poor in all rows on acid soil, despite seed treatment and liming, that the entire experiment was plowed under, although, considering the rate of seeding, the planting on neutral soil would have yielded a normal crop.

Field Results, 1934. In September, 1933, enough ground limestone was added to each of three of the previously limed strips to make the total lime application 10 tons per acre, and enough sugar-factory lime cake was added to the fourth strip to make a total of 20 tons per acre. During the very dry, hot spring of 1934, wind repeatedly destroyed the sparse sugar-beet

TABLE 10.—*Number of sugar-beet seedlings emerged and surviving after 7 days from late (May 26) planting of treated and nontreated seed on limed and nonlimed acid and neutral soil.*

Seed-treatment agent[a]	Acid soil				Neutral soil			
	Nonlimed		Limed		Nonlimed		Limed	
	At emergence	7 days later	At emergence	7 days later	At emergence	7 days later	At emergence	7 days later
Check	262	65	287	120	400	341	405	371
1. C_2H_5HgCl, 2%	333	90	349	135	484	329	465	406
2. $(C_2H_5Hg)_3PO_4$, 5%	311	77	347	145	492	427	463	390
3. $(C_2H_5Hg)_3PO_4$ + hydrated lime	303	71	336	138	437	375	476	437
4. $C_2H_5HgCl + HgCl_2$ + hydrated lime	318	107	350	167	394	352	431	377
5. $(C_2H_5Hg)_3PO_4 + HgCl_2$ + hydrated lime	321	91	345	137	421	373	417	380
6. C_2H_5HgCl + hydrated lime	235	50	284	97	387	313	397	359
7. Hydrated lime dust	270	53	270	102	439	392	424	388
8. Hydrated lime wet roll	287	67	293	124	409	349	413	390
9. Diphenyl mercury, 2%	271	54	318	113	424	379	396	360
10. C_2H_5HgCl + mercury furfuramide	275	61	298	112	424	360	436	409
11. Formaldehyde dust	235	57	294	123	375	309	376	342
12. $(C_2H_5Hg)_3PO_4$ + colloidal phosphate	296	46	346	129	418	355	391	365
Totals	3717	889	4117	1642	5504	4714	5490	4794

[a] Complete formula in table 7.

seedling stands. These climatic factors made emergence and other counts so erratic that virtually no worth-while data were obtained. In general, the results agree with those enumerated for 1933. One set of data, however, may bear analysis.

The data above referred to resulted from an attempt to evaluate by direct macroscopic inspection of seedlings the effectiveness of seed treatment in inhibiting *Pythium de baryanum* under various field conditions.

In the 1934 plantings, which were on the same plots and similar to those made in 1933, an extra check row of 10 hills and a row of seed treated with ethyl mercuric phosphate, 5 per cent, $\frac{1}{2}$ g. per 100 g. of seed, were planted on each plot to be dug up after emergence. Germination was very poor, and, since there was no apparent difference between limed and nonlimed rows, the results from both were added. An attempt was made to classify plants macroscopically as healthy, infected by *Pythium*, or by *Phoma betae*. Such classification was undertaken only after considerable practice in the greenhouse with seedlings infected with known sources of inoculum, observations of which were verified by isolation. After each of the first 2 field

readings, isolations were made to test the soundness of the field classification. A low yield of cultures was obtained, but the only *Phoma betae* observed grew from seedlings classified as infected by *Phoma betae*. Even so, a question mark is in order after the number placed in the "*Phoma*-infected" column in table 11. There seemed to be no *Phoma betae* infection on seed-

TABLE 11.—*Number of healthy and Pythium de baryanum- and Phoma betae-infected sugar beet seedlings grown on acid soil in the field from treated and nontreated seed planted at different dates, 1934*

Date planted	Date counted	Seed not treated			Seed treated		
		Healthy seedlings	Phoma-infected seedlings	Pythium-infected seedlings	Healthy seedlings	Phoma-infected seedlings	Pythium-infected seedlings
April 10	4/30	77	4[a]	5	53	0	0
" "	5/4	40	3[a]	11	54	0	0
" "	5/1	35	1[a]	4	44	0	3
" "	5/4	39	0	4	24	0	0
" 20	5/5	42	0	4	34	0	0
" "	5/5	44	0	4	57	0	1
May 10	5/15	29	3[a]	15	51	0	7
" "	5/17	44	2[a]	8	50	0	5
" "	5/21	6	0	33	47	0	18
" "	5/28	2	0	27	6	0	34
" "	5/15	43	4[a]	5	53	0	0
" "	5/17	37	1[a]	10	51	0	14
" "	5/21	12	0	31	38	0	13
" "	5/28	11	0	23	21	0	24
" 25	6/11	50	0	8	44	0	6
" "	6/14	31	0	30	29	0	35
" "	6/11	63	0	2	38	0	4
" "	6/14	18	0	31	13	0	30
June 11	6/21	34	0	21	35	0	27
" "	6/21	31	0	19	36	0	26

[a] Partially verified by isolations (see text).

lings from the treated seed. The amount of Pythium infection in the early plantings also was very low in the treated rows, and not high in the nontreated rows. In later plantings a larger amount of Pythium infection occurred on seedlings from both treated and nontreated seed.

Field Results, 1935. Several unexpected variables were encountered during the 1935 season. In the anticipated recurrence of severe wind damage, winter rye had been sown between each strip and each replication of the plots previously described. The rye harbored cutworms that had already appeared in the plots in 1934. They destroyed such a great number of beet seedlings, as to make it again impossible to obtain reliable data, other than emergence counts, from these plots.

Furthermore, since no crop rotation had been practiced on the plots, they had been under almost continuous fallow conditions since 1931, when a crop of corn was produced. The 1932 sugar-beet crop failed on acid soil, as did the 1933 and 1934 experimental plantings. During all this time the Pythium infestation in the surface soil apparently had been increasing in

both acid and neutral soil. Somewhat noticeable in 1934, this condition became very striking in 1935. Because of this condition and the prevalence of cutworms, the seedling stand quickly became so meager that it would have been impossible to mature a crop, even on neutral soil. The data obtained (Table 12) largely substantiate those taken in 1933.

TABLE 12.—*Number of emerged sugar-beet seedlings from 2 plantings of treated and nontreated seed on limed and nonlimed acid and neutral soil in 1935*

Seed treatment[a]	First planting (April 5; counted April 30)		Second planting (April 24; counted May 10)			
	Acid soil		Acid soil			
	Nonlimed	Limed	Nonlimed		Limed	
			At emergence	11 days later	At emergence	11 days later
Ck	74	72	28	20	31	22
1	108	139	102	77	104	85
2	126	138	100	79	106	93
3	129	116	80	57	96	81
4	122	110	89	65	123	89
5	130	129	91	66	100	73
6	134	118	49	27	47	30
Total	823	822	559	391	607	473
	Neutral soil		Neutral soil			
	Nonlimed	Limed	At emergence	11 days later	At emergence	11 days later
Total	804	794	708	578	796	694

[a] Formulae in table 14.

The numbers are total numbers of seedlings in 80 hills, from 1 seed ball in each hill. Emergence was greater in the early planting. Liming increased emergence and percentage of survival in the second planting. All seed treatments increased emergence in both plantings, with the possible exception of No. 6 (cuprous oxide) in the second planting.

TABLE 13.—*Number of seedlings from plantings on infested soil with treated and nontreated seed in 1935*

Seed treatment[a]	Average number of seedlings per 10 hills, 2 seed balls planted per hill		
	First planting May 10		Second planting May 28
	At emergence May 27	At emergence May 31	At emergence June 9
Ck	10.3	9.4	3.3
1	25.2	22.2	22.5
2	32.7	29.6	25.9
3	28.1	25.6	20.0
4	26.1	24.6	20.0
5	26.7	24.2	23.1
6	24.6	22.0	9.1

[a] Formulae in table 14.

The data in table 13 were taken from a similar planting on a different plot, also fallowed for one year and almost as heavily infested. The numbers represent average number of seedlings per 10 hills, from 2 seed balls per hill. Here, again, there was greater mergence in the early planting and from treated seed. Cutworm injury made it necessary to abandon this experiment also. In fact, it was necessary to uncover the stubs of damaged seedlings in order to make the emergence counts for the second date of planting.

The seed treatments used in 1935 are listed in table 14.

TABLE 14.—*Sugar-beet seed treatments used in 1935*[a]

Treatment	Toxic agent	Rate of application
1	Ethyl mercuric phosphate, 5 per cent	¼ g. per 100 g. of seed
2	Ethyl mercuric phosphate, 5 per cent	½ g. per 100 g. of seed
3	Combination: Ethyl mercuric phosphate, 5 per cent 25 g. Mercury furfuramide, 5 per cent 75 g.	1 g. per 100 g. of seed
4	Combination: Ethyl mercuric phosphate, 5 per cent 25 g. Merko (3½ per cent metallic mercury) 75 g.	1 g. per 100 g. of seed
5	Combination: Ethyl mercuric phosphate, 5 per cent 50 g. Talc filler 50 g.	1 g. per 100 g. of seed
6	Cuprous oxide	1 g. per 100 g. of seed

[a] See tables 12 and 13.

DISCUSSION

Previous to this study, no one in America had definitely shown that a single pathogen has been the principal cause of damping off of sugar beets in any given locality over a period of years. Peters (19), after his investigations in Germany, clearly indicated that he considered *Pythium de baryanum* the chief cause in what were probably some acid German soils in which the situation was similar to those on which stand failures occur in northern Iowa. Edson (7) included 4 pathogens, *P. de baryanum*, *P. aphanidermatum*, *Phoma betae*, and *Rhizoctonia* sp. as causative agents, but indicates no predominating parasite. It is significant, however, that, when he isolated from beet seedlings grown on Wisconsin soil, his cultures were chiefly *Pythium*, while from seedlings grown on Kansas and Colorado soils he cultured only *Rhizoctonia*; and, as he says of plantings in these latter soils, "A good stand—was secured—in spite of damping-off." Cultures of *Rhizoctonia* have been rarely isolated from diseased beet seedlings from

areas of stand failures in northern Iowa. The writer is not aware that *Rhizoctonia* has destroyed a seedling stand of sugar beets in northern Iowa to the extent that replanting was necessary.

Coons and Stewart (5) also made no attempt to single out one pathogen as consistently more important in causing seedling stand failures, but they did try to differentiate the symptoms brought about by infection with *Pythium, Phoma betae,* and *Rhizoctonia.*

The restriction of severe damage to localized areas within fields leaves little doubt that the pathogen is soil-borne, and almost certainly eliminates *Phoma betae* as a possible causative agent. Seedlings grown in steamed soil from treated *Phoma betae*-infested seed lots have been nearly disease-free. Evidence also has been presented indicating control of seed-borne *Phoma betae* in the field by seed treatment. On the other hand, seed treatment has by no means guaranteed a good stand from late plantings, although it should have been had the principal infective agent been seed-borne *Phoma betae.*

In the writer's experiments, *Pythium de baryanum* has been a much more active parasite than *Phoma betae.* Seed-borne *P. betae* brought about some noticeable injury in steamed soil in the greenhouse if the seedlings were allowed to grow for 2 weeks or more after emergence. *Pythium de baryanum,* in naturally infested field soil, destroyed many seedlings from the same lot before they emerged and many more within a week after emergence. Isolations from field-grown seedlings in early stages of infection and invasion have in most cases yielded cultures of *Pythium,* most frequently *P. de baryanum. Phoma betae* has been recovered occasionally from seedlings grown in the field under low-temperature conditions unfavorable to *Pythium. Rhizoctonia* rarely has been recovered from seedlings grown on areas where stands fail.

Recommendations for control of seedling diseases of sugar beet have thus far been lacking or somewhat indefinite. Arrhenius (1), in addition to noting the abundance of the disease on acid soils, recognized the importance of *Pythium de baryanum* in late plantings. He reports control by liming, but apparently made no date of planting experiments. Edson (7) also records circumstantial observations of severe damage by *Pythium de baryanum* in late plantings in Wisconsin, but, likewise, made no date of planting experiments. Coons and Stewart (5) were concerned primarily with seed treatment as a control measure, but considered their field results were insufficiently consistent to warrant recommendations. In 1937 LeClerg (15) reported his results with Ceresan. At the same time, Reddy and Buchholtz (20), on the basis of the work herein presented, outlined the procedures necessary to improve seedling stands in northern Iowa, namely: plant early with seed treated with 5 per cent ethyl mercuric phosphate; if late planting is imperative, avoid acid, heavily infested fields or areas.

The failure of *Pythium de baryanum* to grow and induce infection rapidly at temperatures not too low for beet-seed germination and seedling

development is a part of the disease picture that is vulnerable to control measures. At soil temperatures below 15° C., Pythium apparently is inactive enough for an adequate seed treatment to check it until the seedlings are too large to be invaded, or, if infection takes place, the invasion is checked before it threatens the life of the young seedling. In 3 successive years (1933–1935) the first plantings of seed treated with 5 per cent ethyl mercuric phosphate produced better stands than any from treated or nontreated seed planted thereafter. Late plantings on acid, heavily infested soil have failed in spite of seed treatment. Generally, any planting made after the first week in May would be a late planting in northern Iowa.

Continued heavy lime dressings have, in time, brought about slight increases in seedling emergence, but not so large as those resulting from earlier planting and seed treatment. The most noticeable effect of lime, even in smaller quantities, has been the improvement in the percentage of survival of emerged seedlings, although in later plantings the effect has not been sufficient to produce a satisfactory stand. Liming by itself has not resulted in sufficient improvement to warrant its expense, but of course, if liming were an established part of a grower's farm program, the effect would be favorable both to stand and yield.

One control practice that has been demonstrated by Coons and Kotila (6), namely, planting on corn land rather than after alfalfa or sweet clover, has been adopted by beet growers in Michigan, but it has not yet produced similar effects for the writer in preliminary experiments in northern Iowa.

SUMMARY

Damping off induces sugar-beet stand failures in northern Iowa under conditions of soil acidity and moderately high soil temperature.

Pythium de baryanum, *Phoma betae* and *Rhizoctonia* are pathogenic to sugar-beet seedlings in the greenhouse. In the field, *Pythium de baryanum* causes over 95 per cent of the damage to germinating and emerging seedlings.

Pythium de baryanum causes a general necrosis of any part of the germinating and emerging seedling and cortical necrosis on slightly larger plants. *Phoma betae* causes a slow cortical necrosis that frequently results in no aboveground symptoms and from which seedlings may recover in field soil. Only a very small percentage of diseased seedlings grown in the field yielded cultures of *Phoma betae*. *Rhizoctonia* has very seldom been isolated from field-grown sugar-beet seedlings.

The *Pythium* isolates taken from sugar-beet seedlings are similar, but not identical. The measurements of their fruiting structures are as follows: Conidia, 10 to 35 μ; oogonia, 16.6 to 22.1 μ; oospheres and oospores, 13.6 to 17.0 μ. Their measurements and cultural characters are like those listed by Butler for *Pythium de baryanum*.

Temperatures below 15° C. were unfavorable to the growth of *Pythium de baryanum* in pure culture and its pathogenicity to sugar-beet seedlings.

Areas in which *Pythium de baryanum* brought about especially poor stands did not differ greatly from areas with good stands in moisture content, but were found generally to be more acid, *e.g.*, below a pH of 6.5. In 1933, a survey of sugar-beet fields in northern Iowa revealed a correlation of 0.745 between soil pH and percentage stand. Two cultures of *Pythium de baryanum* grew uniformly well at pH values of 4.7 to 7.3.

In field experiments liming increased the number of emerging seedlings only slightly, but increased the percentage of survival. Early planting increased emergence and survival. Seed treatment also increased emergence and survival, and was most effective in early plantings.

No single practice has completely controlled damping off of sugar beets. A combination of early planting and seed treatment (5 per cent ethyl mercuric phosphate, 4–7 oz. per 100 lb. seed) can be recommended to improve stands of sugar beets.

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INFECTION OF MAIZE WITH PHYTOMONAS FLACCUMFACIENS, P. INSIDIOSA, P. MICHIGANENSIS, P. CAMPESTRIS, P. PANICI AND P. STRIAFACIENS[1]

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INTRODUCTION

According to Burkholder (1) species of the genus Phytomonas may be divided into several natural groups on the basis of cutural characteristics. One group composed of *P.*[2] *stewarti* (E.F.S.) Bergey *et al.*, *P. flaccumfaciens* (Hedges) Bergey *et al.*, *P. insidiosa* (McCulloch) Bergey *et al.*, *P. michiganensis* (E.F.S.) Bergey *et al.*, and possibly several others has been referred to as the *Stewarti* group, from one of its earliest described species. Another group perhaps not so well defined has been referred to as the *Campestre* group. Remaining species are colorless and may be grouped together on this basis.

Hosts attacked by the organisms of any one group may be widely different. For example, in the *Stewarti* group, *Phytomonas stewarti* primarily causes a wilt of maize (*Zea mays* L.), *P. flaccumfaciens* a wilt of beans (*Phaseolus vulgaris* L.), *P. insidiosa* a wilt of alfalfa (*Medicago sativa* L.), and *P. michiganensis* a wilt of tomatoes (*Lycopersicum esculentum* Mill.). Since these organisms are very similar in cultural behavior, one might expect that a strain of maize very susceptible to *P. stewarti* may also be infected to some extent at least by other organisms of this group or, *vice versa*, *P. stewarti* may infect alfalfa, beans, and tomatoes. This paper is chiefly concerned with such cross-inoculation studies. Effect on maize of *P. flaccumfaciens*, *P. insidiosa*, and *P. michiganensis* of the *Stewarti* group, *P. campestris* (Pam.) Bergey *et al.* of the *Campestre* group, and such colorless forms as *P. panici* (Elliott) Bergey *et al.* causing bacterial stripe of proso millet, *Panicum miliaceum* L., (2) and *P. striafaciens* (Elliott) Bergey *et al.* causing bacterial stripe of oats, *Avena sativa* L., (3) is described. Effect of *P. stewarti* on hosts of certain of the above organisms also is described.

[1] This is part of the research carried on in the Department of Animal and Plant Pathology of the Rockefeller Institute for Medical Research, Princeton, New Jersey, while on a General Education Board Fellowship.

[2] The abbreviation *P.* instead of *Phyt.* is used for *Phytomonas* to conserve space.

MATERIALS AND METHODS

Cultures of *Phytomonas insidiosa*, *P. michiganensis*, and *P. campestris* were obtained from George L. McNew, Department of Animal and Plant Pathology, Rockefeller Institute for Medical Research. Degree of pathogenicity of *P. insidiosa* for alfalfa was not determined. The cultures of *P. michiganensis* and *P. campestris* were found to be fairly pathogenic on Bonny Best tomatoes and Early Jersey Wakefield cabbage, *Brassica oleracea* var. *capitata*, respectively. A culture of *P. flaccumfaciens* was obtained from Florence Hedges, U. S. Dept. Agr., and was found very pathogenic for both Scotia and Golden Cluster beans. Cultures of *P. panici* and *P. striafaciens* were obtained from Charlotte Elliott, U. S. Dept. Agr. These were old stock cultures and proved to be weakly pathogenic for proso millet and Early Pearl oats, respectively.

Two inbred lines of maize were used in testing maize infection with the above organisms. One, GB797, was an inbred line of Golden Bantam sweet corn very susceptible to *Phytomonas stewarti;* the other, OSF, was an inbred line of field corn, very resistant to *P. stewarti.* A detailed description of the genetic resistance or susceptibility of these lines of *P. stewarti* was given in an earlier paper (6). Organisms to be tested on maize were hypodermically injected into week-old seedlings of these two lines. Two punctures per seedling were made: one, at the first node above the mesocotyl; the other, through the leaf sheaths just above the growing point. This method was similar to that used in testing resistance and susceptibility of various inbred lines to *P. stewarti* (6). All tests were made in a greenhouse. Seedlings were grown in 4-inch pots filled with fertile soil, and amply watered. Temperatures were maintained at 80°–90° F. Each test was repeated several times.

To test infection of beans, tomatoes, cabbage, millet, and oats with a strain of *Phytomonas stewarti* highly pathogenic for maize, the following varieties were used: Golden Cluster beans, Bonny Best tomatoes, Early Jersey Wakefield cabbage, proso millet, and Early Pearl oats. These varieties proved to be susceptible to *P. flaccumfaciens, P. michiganensis, P. campestris, P. panici,* and *P. striafaciens,* respectively. Tomato and cabbage plants were started in flats and later transplanted to 6-inch pots. After transplanted plants had become established, inoculations were made by puncturing stems, petioles, and the veins of leaves with a hypodermic needle. Beans were similarly inoculated shortly after emergence. Oats and proso millet were inoculated in seedling stage by a single puncture at the base of the stem.

OBSERVATIONS

Effect of Vascular Parasites of Beans, Alfalfa, Tomatoes, etc., on Maize

Phytomonas flaccumfaciens: First indication of infection of the maize strain, GB797, appeared about 4 days after inoculation as a yellowing of

certain veins of the leaves and a slight discoloration of the parenchymatous tissue on either side. Discoloration of tissue along affected veins was very irregular. Dull-grey areas often appeared intermingled with light and dark green. Brown water-soaked lesions along affected veins, characteristic of infections with *P. stewarti,* did not appear. Very often, however, affected veins became transparent. Later the leaves would split along such veins.

On most seedlings, infection was not severe enough to kill the plants, but, invariably, some of the leaves, unfolded at the time of inoculation, were killed. Often one longitudinal half of a leaf died while the other half remained green. Sometimes, the growing point was killed and the original plant succumbed. Usually, however, such plants developed suckers, which generally were not affected. All infected plants were severely dwarfed.

Microscopic examination of diseased plants showed many of the xylem vessels of the stem, leaves, and mesocotyl filled with bacteria and bacterial slime.

Infection on the strain of maize, OSF, was less severe than on GB797. A few affected veins of the leaf were evident, but dwarfing and mutilation of the plant in general was much less severe (Fig. 1, A and B).

Phytomonas insidiosa: Both OSF and GB797 were affected by this organism, but OSF seemed to be more severely attacked than GB797. Symptoms of infection on the leaves of either host were not numerous. Chlorotic streaks along affected veins occasionally appeared. On one of the inoculated plants of OSF, water-soaked areas of irregular size and shape were noted along one of the affected veins of a leaf.

A common sign of infection of OSF was the drying up of the parenchymatous tissue surrounding the wound made by the inoculating needle through the leaf sheaths just above the growing point on very young seedlings. The plants would often become girdled in this region by a band of dead or dying parenchyma tissues 3–5 mm. wide, and parts above would topple over. Apparently the vascular bundles running through the band of dead or dying parenchyma on the sheath were not seriously affected, for the leaf above or sheath below the affected spot remained normal for a considerable period.

Undisputable signs of infection of OSF were evident upon microscopic examination of the vascular tissues. Figure 2, A, shows a cross section of the mesocotyl of an infected OSF seedling about 1½ inches below the point of inoculation. It may be seen that a considerable number of the vascular elements were plugged or partially plugged. Bacteria could be found in certain vessels of the mesocotyl all the way down to the point of seed attachment. They also were found in certain vessels of the stem, leaves, and leaf sheaths. Figure 2, B, shows a vascular bundle in a leaf sheath in which all the xylem vessels were plugged with a yellow gum-like mass.

Phytomonas michiganensis: GB797 was more readily attacked than OSF. Symptoms of infection on either host were very similar to those obtained with *P. flaccumfaciens* but somewhat less severe. Affected seedlings usually

FIG. 1. Effect of *P. flaccumfaciens* on the inbred lines of corn, OSF and GB797. A. OSF, pot on left, control. B. GB797, pot on right, control. Photographed 10 days after inoculation. C. Effect of *P. stewarti* on Early Pearl oats. Pot on left, control. Photographed 15 days after inoculation.

were dwarfed. The growing point on some was killed, but on such plants suckers readily developed.

Upon microscopic examination of the vascular tissue, many of the vessels throughout the mesocotyl and certain bundles of the stem and leaves were found either completely or partially plugged. Reisolations made from infected veins of leaves were tested on tomatoes. Symptoms of tomato wilt appeared.

Phytomonas campestris: This organism was very reluctant in attacking maize. On OSF no infection was detected. On GB797, the outward signs of infection were manifest largely as dwarfing of the plants. Occasionally, affected veins could be detected on the leaves by a slight discoloration, but such signs were comparatively rare.

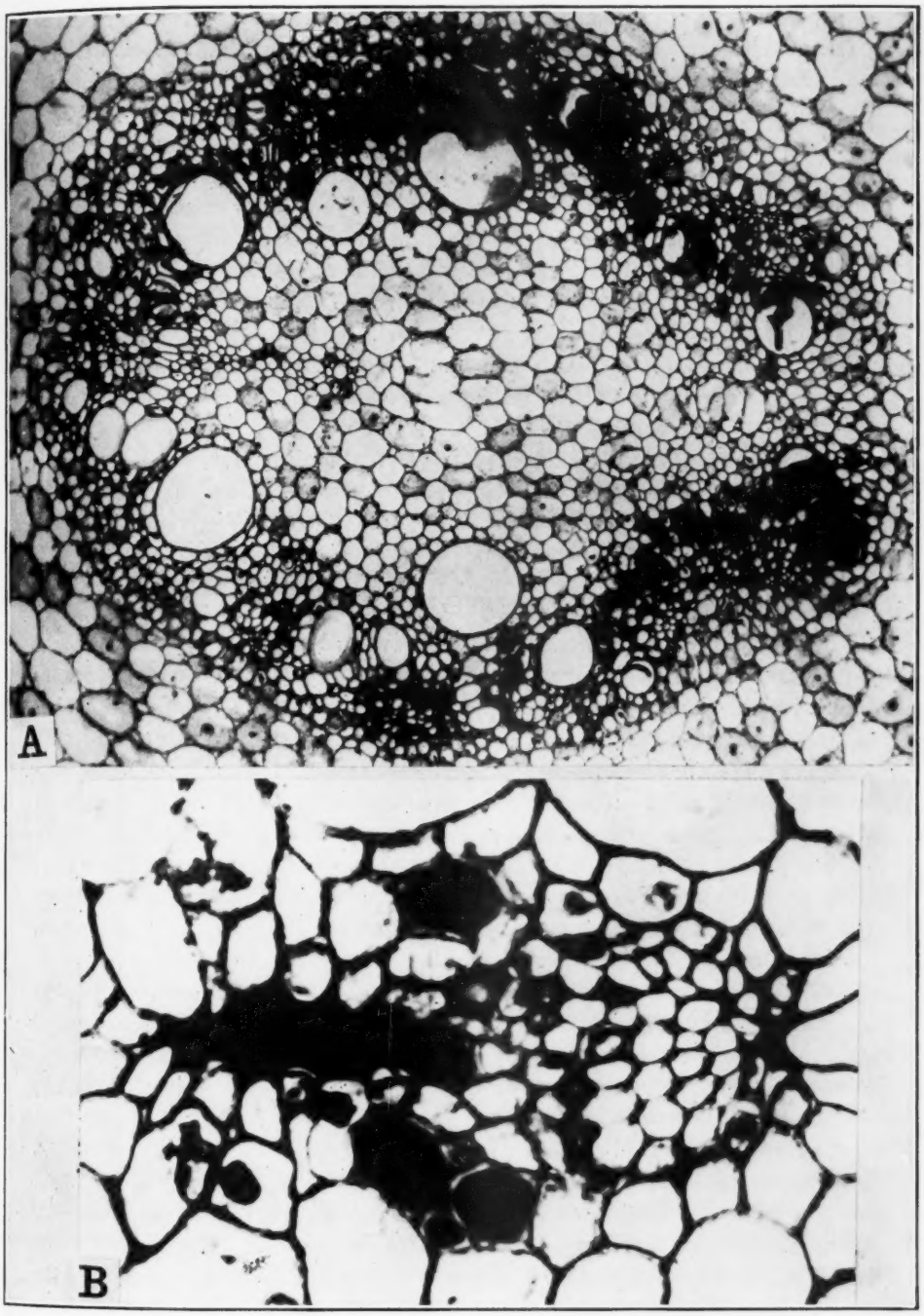

FIG. 2. A. Cross section of the mesocotyl of an infected OSF seedling inoculated with *P. insidiosa*. The yellow gum like masses of bacteria and slime appear black in the photograph. B. Cross section of an infected vascular bundle in a leaf sheath of an OSF plant inoculated with *P. insidiosa*. The xylem elements (dense black areas) were completely plugged.

Upon microscopic examination of the vascular tissues of dwarfed plants a few affected vessels of the mesocotyl and stem were evident. Reisolations made from affected vessels of the stem readily infected and wilted plants of Early Jersey Wakefield cabbage.

Phytomonas panici: Symptoms induced on GB797 by *P. panici* were a little more severe than those produced by *P. campestris.* A slight discoloration of certain veins of the leaves was evident 4–5 days after inoculation. Many of them were bordered on each side by a narrow irregular band of light-green parenchyma. Later, a few small necrotic areas often appeared at irregular intervals along affected veins. Plants were often severely dwarfed. On OSF no infection was detected. Reisolations were made from affected veins of GB797 plants and tested on proso millet. A mild degree of infection, similar to that caused by the original culture, was obtained.

Phytomonas striafaciens: Effect of *P. striafaciens* on GB797 was similar to that of *P. campestris.* The plants were only slightly dwarfed. An occasional vein in the leaves showed a slight discoloration. No signs of infection on OSF were apparent.

Effect of Phytomonas stewarti on Beans, Tomatoes, Cabbage, Proso Millet, and Oats

Fifteen plants of Golden Cluster beans were inoculated with a strain of *Phytomonas stewarti* highly virulent for maize. No outward signs of infection, with the exception of a slight stunting, was evident. Upon examination of the stem tissues four weeks after inoculation, discolored nodes and internodal vessels were found. Reisolations were made from the inter-nodal discolored vessels and their pathogenicity was tested on maize. No change in virulence for maize was noted.

Six plants each of Bonny Best tomatoes and Early Jersey Wakefield cabbage, that were about 3 weeks old, were inoculated with *Phytomonas stewarti.* No infection was evident. The extent of these trials, however, does not justify the conclusion that the plants are immune.

Proso millet was readily infected with *Phytomonas stewarti,* as pointed out previously by Ivanoff (4) and Wellhausen (7). Numerous water-soaked lesions appeared along the veins of the leaves, resembling in manner of development those produced by *P. stewarti* on maize (5). Plants were severely dwarfed but none were killed.

Early Pearl oats also was readily infected with *Phytomonas stewarti.* Lesions on the leaves resembled very closely those produced by this organism on maize. Well-defined semicircular, water-soaked areas appeared along affected veins of leaves 3–4 days after inoculation. These areas often coalesced, forming long, narrow, water-soaked stripes 1–2 mm. wide along the veins. The stripes in time became necrotic and turned light brown in color. Infected plants were usually severely dwarfed but not killed. Figure 1, C, shows a number of oat plants 15 days after inoculation beside controls. Dwarfing of the inoculated plants is evident.

In addition to the above, a variety of soft red winter wheat, thought to be Leap, was tested with *Phytomonas stewarti*. A few water-soaked lesions appeared on the leaves, but, in general, this wheat variety was much less susceptible to *P. stewarti* than was Early Pearl oats.

DISCUSSION AND CONCLUSIONS

In these experiments maize was inoculated with the various organisms when plants were very young and growing rapidly under conditions of high temperature, ample moisture, and fertile soil. It may be argued that, under such conditions, seedlings are very susceptible to mechanical injury and that much of the yellowing and dwarfing observed may have been induced by mechanical injury in the process of inoculation. This possibility, however, may be ruled out, since injury caused by needle punctures in the process of inoculation seemed to have very little if any noticeable effect on the development of the plant as apparent from adequate checks. The yellowing and dwarfing were largely due to the presence of the organism.

It was apparent throughout these investigations that certain of the organisms, namely, *Phytomonas flaccumfaciens, P. insidiosa,* and *P. michiganensis,* grew or multiplied more rapidly in the medium of the corn plant than *P. campestris, P. panici,* and *P. striafaciens.* The first 3 organisms mentioned usually caused considerable injury and often killed the young plants. The last 3 seemed to affect the host very mildly, growing and spreading very slowly at first, but apparently growth soon ceased. It is entirely possible that the mild symptoms brought about by the latter 3 organisms were due not to any special affinity or adaptation on the part of the organism for the host, but to the fact that these young, rapidly growing plants were full of sap and, temporarily, may have contained constituents conducive to a small amount of growth. It is possible that a wide range of unrelated organisms would cause a similar slight dwarfing and yellowing of certain veins of highly turgid seedling plants under optimum conditions for rapid growth.

With *Phytomonas flaccumfaciens, P. insidiosa* and *P. michiganensis,* the situation seemed to be somewhat different. These organisms apparently possessed a greater affinity or were better adapted to the medium of the xylem vessels. This is not surprising, since these organisms appear to be more similar to *P. stewarti* in cultural characteristics than the others.

From the results obtained, it is not contended that corn may be a natural host of *Phytomonas flaccumfaciens, P. michiganensis,* etc., or, for example, that beans may be a natural host of *P. stewarti,* in the sense that these organisms would naturally cross-inoculate or maintain themselves indefinitely on these hosts under field conditions. The results merely indicate that, of the organisms tried, those closely related to *P. stewarti* in cultural characteristics, as determined from artificial media, are also capable of considerable growth in the medium of the corn plant under conditions most favorable to *P. stewarti.* Whether the pathogenicity for maize of *P. flaccumfaciens, P. michiganensis,* or some of the others may be increased by repeated passage

through maize, or whether the pathogenicity of *P. stewarti* for oats or some of the other plants may be increased by successive passages through certain strains of these plants remains to be determined. Studies on these points are under way.

SUMMARY

Effect of *Phytomonas flaccumfaciens, P. insidiosa, P. michiganensis, P. campestris, P. panici* and *P. striafaciens* on maize seedlings, and effect of *P. stewarti* on certain hosts of these organisms is described.

P. flaccumfaciens, P. insidiosa and *P. michiganensis* usually severely injured and often killed inoculated maize seedlings. The others had a more mild effect. Two inbred lines of maize were used in these tests, one (GB797) was very susceptible to *P. stewarti*, the other (OSF) was very resistant to *P. stewarti*. With one exception (*P. insidiosa*), all the organisms tried attacked the line, GB797, more readily than the line, OSF. *P. insidiosa* affected OSF more readily than GB797.

Types of symptoms induced on maize were, in general, very similar. Infection was primarily confined to the xylem elements. Plugging of the xylem vessels of certain vascular bundles in leaves, stems, and mesocotyls of affected seedlings was apparent in varying degree upon microscopic examination several weeks after inoculation. Affected veins in the leaf and tissues adjoining such veins were usually discolored. Very often tissues along affected veins became transparent.

Golden Cluster beans inoculated with *P. stewarti* were slightly dwarfed and showed some discoloration of the nodal and inter-nodal vessels of the stem.

No infection was evident on Bonny Best tomatoes and Early Jersey Wakefield cabbage inoculated with *P. stewarti*.

Proso millet and Early Pearl oats were readily infected by *P. stewarti*. Brown water-soaked irregular stripes, similar to those produced by *P. stewarti* on maize, developed along affected veins.

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STUDIES OF BLACK ROOT ROT OF APPLE[1]

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The studies reported herein are a part of a general study of the black root rot of apple, caused by *Xylaria mali* Fromme, in progress at the West Virginia Station, and are a continuation of studies previously conducted at the Virginia Station by the senior author and others.[3]

I. INFECTION OF STOCKS THROUGH EXPOSURE TO INFESTED SOIL AND DISEASED ROOTS

A study of the invasion of apple roots by *Xylaria mali* under conditions of natural exposure to inoculum in infested soils and diseased roots was made in 2 orchards in Jefferson County, West Virginia. Selected rootstocks were employed and, although none of them exhibited any evidence of resistance, the resultant incidence and intensity of infection are of considerable interest.

The 165 2-year-old trees used in the study were representative of 45 clons that had been propagated from root cuttings of selected seedlings. The seedlings represented 20 open-pollinated varieties of the cultivated apple, *Malus malus*, 15 of American and 5 of European and Asiatic origin; 3 clons of *M. malus* developed by Guy E. Yerkes of the U. S. Department of Agriculture, and 1 clon of *M. zumi*, also originated by Yerkes. The number of individual trees per clon varied from 1 to 20.

The trees were pruned to single whips averaging about 30 inches in height and were set on November 25, 1932, in locations where apple trees had died of black root rot. Areas 10 by 10 feet square were prepared by spading and raking and the trees were set in rows 2 feet apart and were spaced 1 foot apart in the row. All old roots were removed to the depth of the spade. The land was Hagerstown loam, representative of the best of the orchard soils of the county. Four separate locations were used, 3 in the Border orchard, near Kearneysville, and one in the Dutrow orchard, near Charles Town. Each planting will be discussed separately.

Border Orchard

The orchard was about 25 years old, and a number of the original trees had died or were in poor condition because of root rot. Many of the latter showed typical symptoms of black root rot, and occasional stromata of *Xylaria mali* were present.

Site No. 1. The original tree had been removed and was represented by a 4-year-old replant that had died of root rot. All roots, except one, had

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[2] Acknowledgment is made of the services of Dr. L. H. Leonian, who made many of the isolations and furnished cultures for inoculation work.

[3] Fromme, F. D., The black rootrot disease of apple. Va. Agr. Exp. Sta. Tech. Bull. 34. 1928.

been killed and showed typical Xylaria infection. These were removed, and, since no remnants of the roots of the original tree were found, the test on this site was one of infested soil lacking diseased root material other than that that had thoroughly disintegrated.

Forty-six trees were set in this site and, except for one that had died of root rot in 1934, all were alive after 3 seasons' growth. All remaining trees were dug carefully on November 22, 1935, and the dirt was washed from the roots with a hose. Including the tree that had died in 1934, a total of 21 trees showed typical Xylaria root infections, while 25 showed no infection, an infection percentage of 45.7. In some cases infection had been established on the main root; in others main laterals or smaller roots had been invaded, and a number of trees showed 3 or 4 separate lesions. Those with

FIG. 1. Black root rot lesions on the main root and crown of a 2-year old apple tree from Site No. 2. The lesions are indicated by arrows, and the brittle character of the affected wood is shown by the broken tip of the root. Photograph by Dr. A. B. Groves.

severe invasion of the main root and crown doubtless would have died before completion of another season's growth. Typical examples of infection, such as occurred in this and the other plantings, are shown in figures 1 and 2.

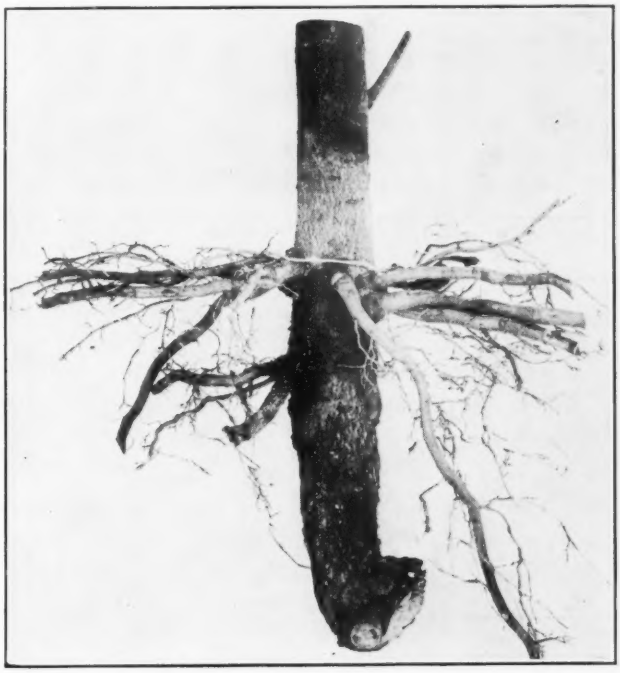

FIG. 2. A 3-year-old tree of *Malus zumi* from Site No. 3. The base of the main root has been completely invaded, and the tree was supported by adventitious roots thrown out above the affected zone. The main root broke off in harvesting.

One of the interesting features of this planting was the localization of the infected trees with reference to the position of the replant and original tree. With one exception all of the infections occurred on one side of this position (Fig. 3). Cases of infection of only one side of the root system of mature trees are rather common, and it would appear in this instance that the original tree had such a one-sided infection and that the fungus persisted in this area, but was not present in appreciable degree in the soil of the other side. Since the root system of the 4-year-old replant was not extensive enough to invade all of the infested area, it also appears that the fungus had persisted there for at least 4 years in the absence of growing apple roots.

Xylaria mali was isolated readily from specimens of root lesions taken from each of the 4 sites included in the study.

Site No. 2. The original tree had been removed in the spring of 1932. The stump, left near the site, and many of the roots remaining in the soil showed typical Xylaria rot. All large roots were removed, chopped into small pieces, scattered over the bed, and worked into the soil with the spade and rake before the young trees were set.

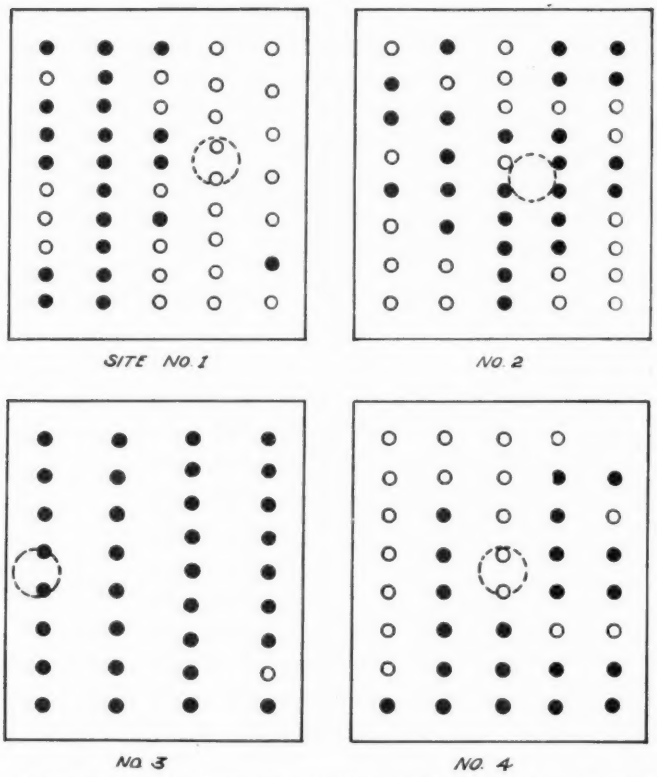

FIG. 3. Diagrams showing location of infected (black circle) and noninfected (open circle) apple trees in 4 experimental plantings. The broken circles show approximate location of the original trees.

The 46 young trees used in this site were all living after 2 seasons' growth. They were harvested November 16, 1934. Twenty-five, or 54.3 per cent, showed Xylaria infection, while 21 were not infected. Of those infected, 11 showed extensive injury, which would seemingly have resulted in death within another year. The main roots were affected, the lesions varying from 2 to 6 inches in length, and the crowns completely girdled. The other 14 trees showed infections on lateral roots only, some bearing only a single lesion, while others bore 3 or 4 separate lesions.

The location of infected and noninfected trees, as shown in figure 3, indicates a more intense infestation in the area adjacent to the center of the original tree than at the borders, and suggests that soil infestation was of greater importance than the scattering of diseased roots.

Site No. 3. This was similar to Site No. 2 in that the original tree had been removed in the spring. The stump left nearby showed that death had followed Xylaria infection. The roots remaining in the soil indicated that infection had occurred chiefly on one side of the root system. The young trees were, therefore, set in this infested area. Affected roots were chopped in small pieces scattered over the area and worked into the ground.

In all, 34 young trees were set and, although all were living after 3 seasons' growth, many were noticeably dwarfed and showed very severe Xylaria infection when harvested on December 4, 1935. Only one tree of the lot showed no infection. Extensive invasion of the main root and crown, as well as some infections of lateral roots, had occurred in 20 trees. The other 13 showed infections on lateral roots only. Ten or more separate lesions were observed in a single root system in some instances. Since all but one of the trees showed infection, no pattern of distribution of the inoculum was apparent. The planting is of particular interest because of the very high (97.1 per cent) incidence of infection.

Dutrow Orchard

Site No. 4. The original trees in this orchard were approximately 30 years old, and a considerable number were missing or were represented by replants of various ages. The young trees were set on the site of an original tree that had been removed a few months earlier but had been left lying at the site. The root system was almost completely invaded by *Xylaria mali* and the organism was readily obtained in pure culture. The old roots were removed from the soil, chopped fine, and distributed in furrows before the young trees were set.

All trees were living at the time of harvest, November 5, 1935. Of the 39 trees, 21, 53.8 per cent, were infected and 18, or 46.2 per cent, showed no infection. Nine of these showed infection on the main root or crown; the others, on main laterals or small roots. One tree was almost dead, the crown having been completely girdled by the fungus.

The diagrammatic representation of this planting shows no evident pattern of distribution of the inoculum.

Although it is known that young apple trees rarely survive to bearing age when set as replants following trees that have been killed by the black rootrot fungus, the plantings reported here supplement this information and reveal the extent and intensity of infection that may occur within 2 and 3 years on young trees planted in thoroughly infested soil.

A summary of the data from the 4 plantings (Table 1) shows that the incidence of infection ranged in the separate plantings from 45.7 to 97.1 per cent and averaged 60.6 per cent. Because of the difficulty involved in har-

Table 1.—*Incidence of infection by Xylaria mali in young apple trees set November 25, 1932, in infested soil*

Site	Period of exposure	Number infected	Number not infected	Percentage infected
1	3 years	21	25	45.7
2	2 years	25	21	54.3
3	3 years	33	1	97.1
4	3 years	21	18	53.8
All		100	65	60.6

vesting and examining complete root systems in the heavy soils employed in the tests, it is probable that a number of lesions on small roots were overlooked and that higher incidences of infection actually obtained.

The intensity of infection varied from lesions on small roots of the current year to extensive invasion of the main root and crown. Many individuals exhibited more than one lesion and in some as many as 10 separate lesions were recorded. Although death of the tree resulted in only one instance, many of the trees were so seriously affected that they would scarcely have survived another season.

The pattern of disease indicated that infection commonly occurs on newly developed roots and progresses from these into older roots and, eventually, into the crown. Death, which results from the complete girdling of the crown, may be delayed by the production of adventitious roots above the affected area. There was no evidence of any toxic effect on the plant as a whole.

While the relative importance of soil and affected roots as sources of inoculum cannot exactly be defined, from these studies, it is evident that soils may carry inoculum in the absence of visible root material.

No evidence of any promising measure of resistance was found among the 44 clons of *Malus malus* or the single clon of *M. zumi*. Absence of infestion is attributed to lack of contact with inoculum rather than to disease resistance.

II. SUSCEPTIBILITY OF STOCKS TO PURE CULTURE INOCULATION

In addition to the plantings in infested soil, inoculation tests with *Xylaria mali* in pure culture were made on a group of 23 selected rootstocks of *Malus*. All of these are included in stock-scion studies now in progress at the University Experiment Farm, Kearneysville, West Virginia, the studies being cooperative between the West Virginia Agricultural Experiment Station and the Division of Horticultural Crops and Diseases of the Bureau of Plant Industry, U. S. Department of Agriculture.

The trees used in our tests were remnants from the stock-scion planting and included 12 clonal stocks, 5 originated by the East Malling Research Station, and 7 by the United States Department of Agriculture, and 11 seedling stocks of open-pollinated varieties. The stocks had been budded to the varieties Gallia, Jonathan, Red Rome, Starking, Staymared, and York; but, as there was no evidence of a scion influence on their susceptibility, they will be treated in this report as though a single scion variety had been used. All material, except East Malling stocks 1, 3, and 13, was supplied by the cooperating federal agency. The exceptions were grown and budded by the Department of Horticulture of the West Virginia Station.

A total of 465 trees, of vigorous 1-year whips on 2- or 3-year roots, were inoculated with a mixture of 8 isolants of *Xylaria mali*, grown in pure culture on steamed rice, and planted on March 31, 1933, in Hagerstown loam at the Kearneysville experiment farm. They were spaced 30 inches apart

in rows 12 feet apart. The inoculum was applied as a plaster on a small area of a main lateral root from which the bark had been scraped, and was held in position with a narrow band of cloth.

The soil was quite dry when the trees were set, and a rather prolonged period without rainfall ensued. These conditions were evidently unfavorable for infection, since only 26 of the 465 inoculated trees became infected. Five of these died after 2 seasons of growth and the others were living when harvested in November, 1935. Twenty-seven other trees died from causes other than black root rot.

The inoculation was repeated on June 7, 1935, with a pure culture of *Xylaria mali*, designated as 1–I in our series and isolated from a diseased York Imperial apple tree, near Kearneysville. A total of 86 apple trees of the planting used in the preceding test, now 2 years old, were inoculated *in situ*. The soil was removed until a side root, of about the diameter of a pencil, was exposed. This was scraped lightly with a knife, the inoculum applied, and the soil replaced. Only vigorous, healthy trees were employed. Soil conditions at the time and for some weeks thereafter were excellent for the establishment of infection, as there was abundant rainfall.

TABLE 2.—*Susceptibility of rootstocks of Malus to Xylaria mali. The figures in parentheses show numbers of infected trees that died of root rot*

Clonal and seedling stocks	Inoculations of 3–31–1933 Number		Inoculations of 6–7–1935 Number	
	Inoculated	Infected	Inoculated	Infected
Clonal stocks				
East Malling, Type 1	23	3	7	7
" 2	27	1	3	3
" 3	8	0	3	3
" 13	17	0	3	1
" 15	12	1	3	3
U.S.D.A. Type 200	4	0	1	1
Vermont 313	19	0	5	5
316	42	5(1)	3	3
317	18	1(1)	3	3
323	21	2	3	3
Minnesota 329	60	7(1)	3	3
Malus zumi	4	2(2)	2	2
Seedlings stocks				
Delicious	29	0	6	6
Fameuse	24	0	6	6
French Crab	14	0	3	3
Grimes	9	0	3	3
Jonathan	7	0	3	3
McIntosh	37	2	7	6
Northern Spy	31	1	7	5
Rome	19	1	4	4
Tolman	9	0	3	3
Wealthy	14	0	5	5
Winesap	17	0	5	5
Total	465	26(5)	91	86
Per cent		5.6		95.5

The trees were harvested 171 days after inoculation on November 17, 1935. Of the 91 inoculated trees 86, or 95.5 per cent, showed infection. The inoculum was readily recovered in pure culture in a number of tests. The infection had progressed in nearly all instances from the point of inoculation on the small lateral root into the crown, and in a few cases the crown had been almost completely girdled. Many of these crowns ranged between 2 and $2\frac{1}{2}$ inches in diameter. The rapidity and extent of the invasion and the high incidence of infection were particularly noteworthy. They afforded ample proof of the vigorous pathogenicity of the fungus under the conditions of the experiment. As noted previously the trees had been established under good conditions for growth 2 years prior to inoculation.

The response of the different rootstocks is presented in detail in table 2. The low incidence of infection resulting from the first series of inoculations warrants no conclusions as to varietal susceptibility; but the second series indicates very clearly that no one of them exhibits any measure of resistance to the strain of the fungus employed.

SUMMARY

High incidence of black root rot occurred within 2 and 3 years on young apple trees planted in thoroughly infested orchard soils. Of 165 trees under exposure, 100 (60.6 per cent) developed infection ranging from single lesions to as many as 10 separate lesions, and extensive invasion of the roots and crowns.

No evidence of any promising measure of resistance was exhibited by any one of 45 clons of *Malus* spp. exposed to natural infection, or by any one of 12 clonal stocks and 11 seedling stocks inoculated with *Xylaria mali* in pure culture. In one series of inoculations, 86 of 91 trees (95.5 per cent) became infected.

CONTACT PERIODS IN GRAFT TRANSMISSION OF PEACH VIRUSES

L. O. KUNKEL

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INTRODUCTION

None of the peach-virus diseases of the United States have been transmitted mechanically except by some form of grafting. Yellows, little-peach, rosette, and mosaic viruses are readily passed by either buds or scions. It is generally recognized that, in order to get transmission, grafted tissues must unite with the tissues of the stock. Buds and scions that die usually fail to infect. However, when they do not succumb promptly following the budding and grafting operations, they occasionally transmit disease. Insofar as the writer is aware, the period of contact required for transmission by means of buds or scions has not been reported for any plant virus. The question as to how long transplanted peach tissues must remain in contact with healthy tissues in order to obtain transfer of virus arose in connection with studies on the 4 diseases mentioned above. The further question as to whether or not each of the viruses would require the same period of contact was also considered. Experiments made for the purpose of attacking these problems have been completed and are reported below.

METHODS

Two methods were employed in attempts to determine the contact period necessary for transmission of peach yellows. In a few preliminary experiments both buds and scions were used in making inoculations. Since buds were easier to transplant than scions, and since nearly all buds lived, while an occasional scion died from mechanical injuries received when trees were watered, the former were employed in subsequent experiments.

The first method to be given a trial was the following: A diseased bud was inserted in the stem of each of several trees at a point about 6 inches above the soil level. After varying periods of time, the stems were cut off just below the inserted buds. As it was desirable to cut the stems close to the buds, but important that none of the infected bud tissues should be left on the stumps, each cut stem was examined to determine whether or not the nose of the bud had been clipped accidentally. In case the bud had been severed, a small portion of the stump was cut off. The trees were usually cut about 1 mm. below the transplanted tissues. After some days sprouts grew from the stumps. If virus had been transmitted, the sprouts showed the symptoms of yellows; if not, they were normal in appearance. In order to obtain transmission, it was necessary for virus to pass into the tree and move down the stem to a point below that at which the cut was made. After using this method in several experiments, it was abandoned because stumps occasionally died without producing sprouts.

The method finally adopted was to insert diseased buds in the stems of healthy trees, leave them for different periods of time, and then remove them. No procedure was found by which it was possible to remove completely the implanted bud tissues without injury to adjacent stock tissues. Removal of buds was accomplished in the following manner. The blade of a knife was used to lift the tail of the bud sufficiently to permit its being grasped by the thumb and forefinger. The bud was then stripped from its position by a sudden jerk. The bark that had covered the bud was cut off and the portion of the stem that had been in contact with the bud was scraped with a knife. This was done in order to remove any bits of bud tissue that might have adhered either to the bark or the stem of the tree. These operations destroyed all of the tissues that had been in immediate contact with the bud. Any bud causing infection had to remain in place long enough to allow the passage of virus into tissues that were not removed by the knife.

All buds were wooded and those used in any particular experiment were taken from the same tree. Since healthy and diseased meristematic tissues were pressed tightly together over the area of the stem covered by the bud, it was expected that the contact period required for transmission would be the same, or approximately so, in different trees and in different experiments. When this expectation was not realized in tests by means of single-bud inoculations, 5 buds and later 10 buds were inserted in each tree. However, the multiple-bud method of inoculation did not materially improve uniformity of results and was soon discontinued.

Potted seedling peach trees of about the same size and in the same state of vigor were used in each experiment. The ages of the trees in different experiments varied from 6 months to 2 years, but those used in any given experiment were of the same age. Only trees that were in prime condition for budding were inoculated. The same procedure was used in determining the contact periods necessary for transmission of little-peach, rosette, and mosaic viruses. All of the viruses were from sources metioned in previous papers.[1, 2]

EXPERIMENTAL RESULTS

Experiments to determine the contact period necessary for transmission of yellows were begun in January, 1930. Trees that had started growth following a dormant period in cold frames were used. In the first experiment 5 lots of 10 trees each were inoculated with yellows buds. One tree in each lot was cut off immediately below the point of inoculation each day for a period of 10 days following the budding operation. None of the trees cut off within 4 days after budding were infected. Two of the 5 trees cut 5 days after budding and 4 of the 5 cut 6 days after budding became diseased.

[1] Kunkel, L. O. Immunological studies on the three peach diseases, yellows, rosette, and little peach. Phytopath. **26**: 201–219. 1936.

[2] ———. Peach mosaic not cured by heat treatments. Am. Jour. Bot. **23**: 683–686. 1936.

All that were cut 7, 8, 9, and 10 days after budding took yellows. Trees from one of the sets are presented in figure 1. The experiment showed that yellows virus did not pass from diseased buds into tissues below the points at which the trees were cut in as short a period as 4 days, that it sometimes passed in 5 days and that it usually passed in 6 or 7 days.

FIG. 1. Ten peach seedlings inoculated with yellows buds and cut off below points of insertion of buds after varying periods of time. Trees in the upper row were cut 1, 2, 3, 4, and 5 days after inoculation; trees in the lower row 6, 7, 8, 9, and 10 days after inoculation. The trees cut 1, 2, 3, 4, and 6 days after inoculation remained healthy; all others became diseased, although the second and third trees in the lower row did not show marked symptoms when the picture was taken. Virus passed into the last tree in the upper row after a contact period of 5 days.

In further experiments made later in 1930, longer contact periods were required. In several instances virus failed to pass during periods of 10 days. Seventeen different experiments, similar to the experiment described

above, but differing in that contact periods were terminated by removing the transplanted buds rather than by cutting the trees and differing in the number of trees used in some instances, have been made since 1930. The results obtained show that the contact periods necessary for transmission vary with the season of the year and with the age and condition of the trees inoculated. The shortest periods were obtained in early spring when trees were growing fastest. As the season advanced, the periods lengthened. In only one instance was yellows transmitted during a contact period as short as 3 days. The tree giving this minimum period was inoculated on February 18. In an experiment started February 20, transmission was obtained in 1 tree following a contact period of 4 days. On several occasions transmission was secured following contact periods of 5, 6, and 7 days, but longer intervals usually were required. Fairly uniform results were obtained with trees of the same age inoculated on the same date, but, even after the results of many experiments were known, it was not possible to predict accurately what the minimum period of contact would be for any given set of trees. However, the approximate length of the period was predictable. Under the favorable growing conditions of early spring, yellows virus always passed in from 3 to 7 days. At other seasons of the year it usually required from 8 to 14 days.

Results with little peach were similar to those with yellows. This virus usually required from 8 to 14 days to pass from little peach buds into healthy trees. The shortest period that permitted transmission was 5 days. After evidence was obtained that yellows and little-peach viruses are closely related,[3] experiments with the latter were discontinued.

The results with rosette closely paralleled those with yellows and little peach. In an experiment started February 19, transmission was secured after a contact period of 4 days. In 1 of 2 other experiments started on the same date, the minimum period was 5 days. Much longer contact periods usually were required.

The results obtained with yellows, little-peach, and rosette viruses showed that each needed about the same period of contact for transmission. They usually required about 5 days in early spring and about 10 days at other seasons of the year.

Peach-mosaic virus was first used in experiments carried out in 1936. In early spring it frequently was transmitted after contact periods of 2 days. This was shorter than the shortest contact periods that gave transmission of yellows, little peach, and rosette. At other seasons of the year, mosaic always was transmitted after much shorter intervals than the other viruses. However, it never passed in less than 2 days.

The difference in the behavior of mosaic virus and yellows and rosette viruses was most striking in tests carried out during seasons of the year when the latter required rather long periods of contact. The results of 3 experiments with the 3 viruses are presented in table 1. The first of the experi-

[3] See footnote 1.

TABLE 1.—*Contact periods required for transmission of yellows, rosette, and mosaic viruses*

Contact periods in days	Experiment 1			Experiment 2			Experiment 3		
	Trees budded with			Trees budded with			Trees budded with		
	Yellows	Rosette	Mosaic	Yellows	Rosette	Mosaic	Yellows	Rosette	Mosaic
1	−	−	−	− − − −	− − − −	− − − −	− − − − −	− − − − −	− − − − −
2	−	−	−	− − − −	− − − −	− * * −	− − − − −	− − − − −	− − − − −
3	−	−	*	− − − −	− − − −	* − − * *	− − − − −	− − − − −	* − − − * −
4	−	−	*	− − − −	− − − −	* * * *	− − − − −	− − − − −	* * − − * *
5	−	−	*	− − − −	− − − −	* * * *	− − − − −	− − − − −	* * * * −
6	−	−	*	− − − −	− − − −	* * * *	− − − − −	− − − − −	* * * * *
7	−	−	*	− − − −	− − − −	* * * *	− − − − −	− − − − −	* * * * *
8	−	−	*	− − − −	− − − −	* * * *	− − − − −	− − − − −	* * * * *
9	+	−	*	− − − −	− − − −	* * * *	− − − − −	− − − − −	* * * * *
10	−	−	*	− − × −	− − − −	* * * *	+ + + + +	× × × × ×	* * * * *
11	−	−	*	+ − + +	− − − −	* * * *	+ + + + +	× × × × ×	* * * * *
12	−	−	*	+ + + +	− − − ×	* * * *	+ + + + +	× × × × ×	* * * * *
13	+	×	*	+ + + +	× × − ×	* * * *	+ + + + +	× × × × ×	* * * * *
14	+	×	*	+ + + +	× × × ×	* * * *	+ + + + +	× × × × ×	* * * * *
15	+	×	*						
16	+	×	*						
17	+	×	*						
18	+	×	*						
19	+	×	*						
20	+	×	*						

+ = yellows infection.
× = rosette infection.
* = mosaic infection.
− = no infection.

ments was started on April 18, the second on June 22, and the third on September 7. In each of the experiments mosaic required much shorter periods of contact than either yellows or rosette. In the first, mosaic was transmitted after 3 days, while yellows and rosette were not regularly transmitted in less than 13 days. In the second experiment mosaic was transmitted to 2 trees with contact periods of 2 days, to 3 trees with contact periods of 3 days, and to all trees with contact periods of 4 days or longer, while yellows and rosette required from 10 to 14 days. In the third experiment mosaic was transmitted to 2 trees with contact periods of 3 days and to all trees with contact periods as long as 6 days, while both yellows and rosette required 10 days.

DISCUSSION

The experiments reported in this paper show that yellows, little-peach, and rosette viruses usually were transmitted only after rather long periods of contact between diseased and healthy tissues. Periods of about 10 days were required during all seasons of the year except early spring. The minimum periods obtained by early spring budding were 3 days for yellows, 5 days for little peach, and 4 days for rosette. The differences in the lengths of the minimum periods are believed not to be significant, since each of these viruses usually passed in about the same period of time.

While the minimum period of contact necessary for the transmission of mosaic was only 1 day shorter than the corresponding minimum period found for yellows, the difference is believed to be significant. Under the same or similar conditions, mosaic was always transmitted after shorter periods, and usually after much shorter periods, than were the other viruses.

The suggestion frequently has been made that viruses may pass from cell to cell through plasmodesmata.[4] It was thought that the period of contact required for transmission of a virus might represent the time necessary for the establishment of plasmodesmata between diseased and healthy cells. If this were true, it would seem that, under similar conditions, the 4 peach viruses should require about the same periods of contact. The fact that yellows, little-peach, and rosette viruses usually required 4 or 5 times as long as mosaic virus to pass from diseased buds into healthy stems suggests that plasmodesmatal connections may be made more quickly between healthy and mosaic-affected tissues than between healthy and yellows-affected tissues, healthy and little-peach-affected tissues, or healthy and rosette-affected tissues, but this may not be the correct explanation. Some property inherent in the mosaic virus may account for the short contact period required for its transmission.

SUMMARY

Contact periods required for transmission of the viruses causing yellows, little peach, rosette, and mosaic of peach by budding are reported. Mosaic

[4] Livingston, L. G. The nature and distribution of plasmodesmata in the tobacco plant. Am. Jour. Bot. 22: 75–87. 1935.

virus usually passed in from 2 to 3 days, the other viruses in from 8 to 14 days. If transfer of mosaic virus depends on the development of plasmodesmatal connections between diseased and healthy tissues, these connections must sometimes be established between mosaic and healthy tissues within a period of 2 days.

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DEVELOPMENT OF A DIFFERENTIAL INOCULATION TECHNIQUE FOR DIPLODIA STALK ROT OF CORN[1]

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(Accepted for publication March 8, 1938)

Among the most important disease problems of dent corn, *Zea mays* L., is a complex of stalk disorders commonly referred to as stalk rot. As implied in the preceding sentence, the term stalk rot is an inclusive one from the standpoint of causal relationships. From the standpoint of symptoms or ultimate effects the term has, however, a rather definite meaning to plant breeders generally, and is used in reference to stalk breaking when it occurs below the ear. The resultant lodging is distinguishable from that caused by poor root anchorage or root diseases.

The modern method of corn improvement, which involves hybridization among inbred lines, offers the most practical means for the control of the stalk rots through breeding for resistance to these diseases. That considerable progress has been made in the control of stalk rots is evidenced by the minimum of stalk breaking observed in many hybrids now in commercial production. In the development of their inbred lines, breeders have discriminated severely against stalk breaking, with the result that the relatively high level of resistance to stalk rot attained in the recombinations is a common and distinguishing feature of hybrid corn generally. This is a characteristic to which must be credited in no small measure the reason for the general popularity of hybrid corn.

A rapid method of determining relative resistance to stalk rot would be very useful to the corn breeder, and would in all probability stimulate more direct attention to this important disease problem in the breeding practices. A rapid method of measuring relative resistance also would facilitate needed studies on the nature of resistance and on other fundamental aspects.

[1] Received for publication Feb. 25, 1938; issued July 15, 1938. Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, the Wisconsin Agricultural Experiment Station and Funk Bros. Seed Co.

[2] The writers are indebted to Mr. Eugene H. Herrling, of the Department of Plant Pathology, University of Wisconsin, for the drawing in figure 1.

The parasitic relationships involved in the stalk-rot complex have not been clearly defined. Of the various fungi believed to be directly associated with stalk rots, *Diplodia zeae* (Schw.) Lév. probably is of greatest importance under Illinois conditions. The rôle of *D. zeae* as a primary parasite causing stalk rot and stalk breaking has been established from adequate inoculation experiments.

The data presented herein were obtained in an artificial inoculation experiment conducted near Bloomington, Illinois, in 1931, and concern initial studies on the development of a differential inoculation technique for the Diplodia stalk rot of corn.

MATERIALS AND METHODS

Thirteen single crosses representing various combinations among 9 different inbred lines of dent corn were used. The hybrids were planted across a strip of new-plowed virgin prairie soil in drilled rows about 60 feet long. The individual plants were spaced about 1 foot apart in the rows. The first replication consisted of 2-row plots, while additional replications of the hybrids were planted in single rows. The plants in the first row of the 2-row plots were artificially inoculated and those in the second row were reserved for notes on stalk rot resulting from natural causes or natural sources of infection. The additional single-row replications were needed to provide the larger populations of noninoculated plants believed necessary to obtaining data on stalk rot from natural sources and also for additional inoculations in certain of the hybrids, as will be explained later.

Resistance to stalk rot is relative, and varying degrees of resistance or of susceptibility usually may be found within any arbitrary grouping of lines. This holds in the following classification for the 9 inbreds involved in the hybrids studied in this experiment: Resistant, *Hy;* susceptible, *Lan, R313,* and *H;* intermediate, *B, 90, A48, A,* and *L.* The classification of these inbreds is based on the reactions of the lines themselves to stalk rot from natural causes as observed in former years.

Inoculations were made during the last week in August, or about 3 or 4 weeks after the pollination period, in the following manner. A puncture was made through the center of a lower internode, usually about 1 foot above the soil line, with a steel needle about 2 millimeters in diameter. Inoculum consisting of a water suspension of pycnospores[3] from a monospore culture of *Diplodia zeae* was then injected into the puncture by means of a syringe having a glass-tube outlet.

Final notes were taken over a period of several days during the second week in October. Data taken on the inoculated plants involved 2 different measurements of disease, (1) pith spread, and (2) cortical spread.

Pith Spread. Determined after splitting the stalks longitudinally with a knife and then measuring the linear distance of spread of the rot from the

[3] Cultures grown on oatmeal agar incubated for about 6 weeks at room temperature produced abundant pycnidia.

point of inoculation. The pith readings were calculated in terms of the percentage of the distance between the base of the plant and the first ear.

Cortical Spread. A measure of the surface area of the outer cortex visibly rotted or discolored. The cortical readings also were expressed in terms of the percentage of the surface area between the base of the plant and the first ear.

Data taken on the noninoculated plants included (1) percentage of plants whose stalks showed evidence of natural infection with stalk rot; and (2) percentage of plants with stalks broken beneath the point of ear attachment. Thus, 4 different measurements of amount of disease, or of relative resistance, were obtained for each of the 13 hybrids; pith spread and cortical spread for the inoculated plants, and natural infection and broken stalks for the noninoculated controls. The correlations among the results from these different methods of measuring disease are used as the criterion for judging the efficiency of the artificial inoculation method as a means for determining relative resistance.

All badly smutted, barren, and otherwise undesirable plants in both the inoculated and the noninoculated series were discarded from consideration when final notes were taken. All plants in the artificially inoculated rows whose stalks showed evidence of natural infection with *Diplodia zeae* or other stalk rot fungi, likewise were discarded. Since the mortality from this source was expected to be high, especially in susceptible hybrids, an additional number of inoculations were made in some of the replicated plots to insure sufficiently large populations.

TABLE 1.—*Reactions of 13 dent corn single crosses to stalk rot from natural sources of infection, and to stalk rot following artificial inoculation with Diplodia zeae, at Bloomington, Ill., 1931*

Hybrid	Noninoculated				Inoculated		
	Natural infection		Broken stalks		Number of plants	Pith spread[a]	Cortical spread[b]
	Plants examined	Plants infected	Plants examined	Plants broken			
	No.	*Per cent*	*No.*	*Per cent*			
A × Hy	32	3.1	85	7.1	32	14.1	1.9
Hy × L	30	6.6	55	7.3	46	23.5	3.2
H × Hy	25	4.0	123	5.6	30	17.4	4.4
B × Hy	6	16.7	8	0.0	19	39.9	8.6
B × 90	45	15.5	65	7.6	45	37.6	11.9
A × L	31	45.1	90	28.9	50	33.6	12.0
A48 × L	29	3.4	117	10.2	44	28.1	12.3
B × H	31	32.2	46	39.1	23	32.3	15.7
90 × R313	26	23.1	128	44.5	45	41.6	27.8
Lan × 90	30	30.0	83	28.9	45	58.9	39.5
H × 90	31	51.6	80	50.0	50	53.9	43.7
Lan × H	33	69.7	61	59.0	46	58.0	45.5
Lan × R313	32	81.2	88	69.3	47	74.6	68.6

[a] Linear measurement of pith tissues rotted are in terms of the percentage of the distance between the base of the plant and the first ear.

[b] Surface area of the cortex rotted as observed from external appearances are in terms of the percentage of the total surface area between the base of the plant and the first ear.

RESULTS

The data for both the inoculated and the noninoculated populations of the 13 single crosses studied are summarized in table 1.

Correlations Between Results from Inoculated and Noninoculated Populations

The data in table 1 show that wide differences in reaction obtained among the hybrids in both the inoculated and the noninoculated series. In order that the comparison of the results between the inoculated and the noninoculated series may be facilitated, the data for each hybrid are reassembled and

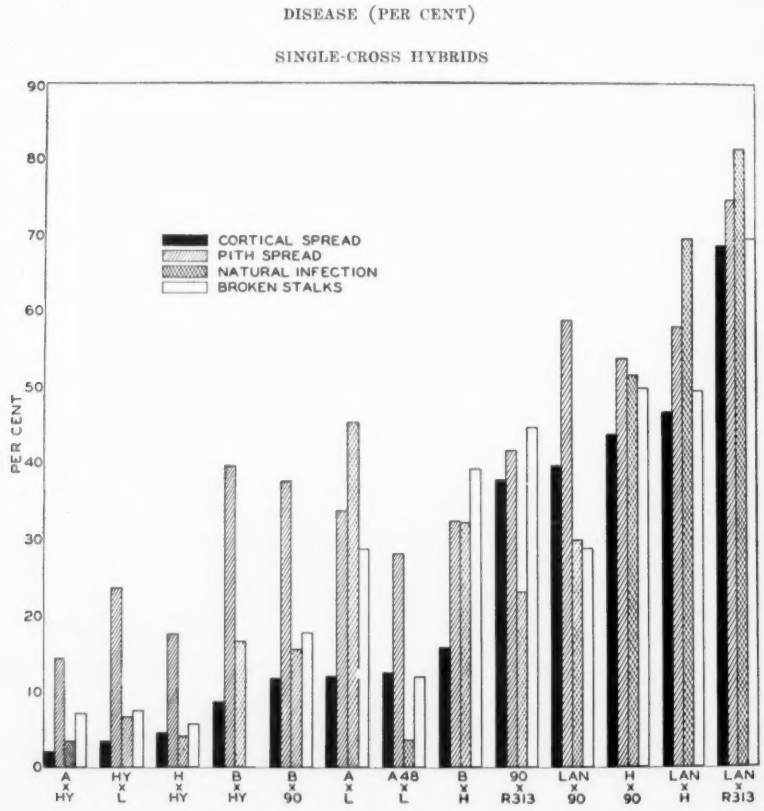

FIG. 1. Comparative amounts of stalk rot in noninoculated plants and in plants artificially inoculated with *Diplodia zeae* in each of 13 single crosses of dent corn. Cortical spread and pith spread are measurements of stalk rot for the inoculated plants; natural infection and broken stalks are measurements for the noninoculated plants. (Data in table 1.)

shown graphically in figure 1. The correlation coefficients are given in table 2.

The correlation between pith spread and cortical spread (inoculated plants) is +0.948 and between natural infection and broken stalks (noninoculated plants) is +0.909. These high, positive correlations indicate

TABLE 2.—*Correlation coefficients between various measurements of amount of stalk rot in inoculated and in noninoculated populations of 13 single-cross dent corn hybrids*

Character	Pith spread	Cortical spread	Broken stalks
Cortical spread	+0.948		
Broken stalks	+0.821	+0.899	
Natural infection	+0.853	+0.878	+0.909

there was little difference between the measurements of disease for the respective populations. Comparing the results from the inoculated series with those from the noninoculated series it is seen in table 2 that the correlations between pith spread and broken stalks, and between pith spread and natural infection are +0.821 and +0.853, respectively. The correlation between cortical spread and broken stalks is +0.899; and between cortical spread and natural infection is +0.878. It is apparent from the magnitude of these correlations that the results from the artificially inoculated series approximated very closely those obtained from the noninoculated controls. Stated differently, the relative resistance of the hybrids studied in this experiment was measured to a satisfactory degree of accuracy by means of artificial inoculation.

Influence of Inbreds upon Hybrid Reaction

The positive influence of inbreds upon hybrid reaction is illustrated in the results obtained (Fig. 1). The 4 different hybrids having the resistant

FIG. 2. Hybrid H × 90 following inoculation of the stalks with *Diplodia zeae*.

inbred *Hy* as one of the parents ranked highest in resistance for cortical spread. In contrast, the 2 hybrids that combined 2 susceptible inbreds ranked lowest in resistance. The 1 hybrid involving a highly resistant and a very susceptible inbred ($H \times Hy$) reacted resistant, indicating a dominance of resistance in this cross. Field photographs illustrating typical reactions of resistant and of susceptible hybrids, to artificial inoculation and to stalk rot from natural sources, are shown in figures 2 to 4.

FIG. 3. The same hybrid as in figure 2 (row in left foreground) noninoculated. Note the evidence of this hybrid's susceptibility to stalk rot as indicated by its reaction to artificial inoculation, and here by the large number of broken stalks which resulted from natural causes or natural sources of infection. Compare with photograph in figure 4.

DISCUSSION

The most promising aspect in the results from the inoculation studies described is the evidence that reaction to Diplodia stalk rot can be measured by means of artificial inoculation. The results obtained also indicate that reaction to stalk rot is hereditary in nature. These facts are of basic importance to the problem of the development of a useful differential inoculation technique for breeders.

A promising lead for studies on the nature of resistance is suggested by the differences shown in the ability of the hybrids to restrict the advance of the fungus in their stalk tissues. Preliminary histological studies by the senior writer[4] showed rather striking differences between a hybrid having

[4] Smith, A. L. The reactions of inbred and hybrid strains of yellow dent corn to infection by *Diplodia zeae* (Schw.) Lév. (Unpublished thesis) 1932.

FIG. 4. Hybrid $H \times Hy$ following inoculation of the stalks with *Diplodia zeae* (left) and its reaction to stalk rot from natural sources of infection (right). The influence of the resistant inbred *Hy* is shown in the comparison of the photographs in figures 2 and 3.

the resistant inbred *Hy* as one of its parents, and one having the susceptible inbred *Lan* as a parent, with respect to the relative amounts of sclerenchyma tissues and the thickness of cell walls in parenchymatous tissues. The studies are considered too limited for any general conclusions but, nevertheless, are very suggestive.

The data presented herein on the reactions of the single-cross hybrids represent results from but a single experiment and are taken from the unpublished thesis referred to above. It should be stated, however, that in the course of the thesis studies in experiments in which the matter of method of inoculation was stressed particularly, a very considerable amount of observational evidence was obtained that resistance to Diplodia stalk rot can be measured by means of artificial inoculation. Additional and quite conclusive evidence has since been obtained by the junior writers in subsequent studies involving the application of the artificial method of inoculation to special problems.

It was realized at the time these studies were completed that the accomplishment of the differential inoculation technique marked a good beginning, but that many studies yet remained to be made before the method could be recommended for practical application. At the present time the inocu-

lation problem has resolved itself into a number of specialized studies. Among things to be considered, the relationship between Diploidia stalk rot and other stalk rots is of utmost importance. How extensively does Diploidia stalk rot occur? Would resistance to Diplodia stalk rot mean resistance to other stalk rots; and, if this should prove to be true, will it be possible to use *Diplodia* in artificial inoculations as a general tester for stalk rot resistance? Another very important problem concerns possible relationships between susceptibility to stalk rot and factors for yield. The questions raised must be answered with definite information in order to know just when and where the inoculation method may be used to advantage. There also remain problems in technique, such as those pertaining to size of population, stage of maturity for inoculating, and others that likewise must be investigated before the artificial method for evaluating resistance may be considered as a usable tool in the breeding program.

SUMMARY

Data are presented from studies on the development of an inoculation technique for the Diplodia stalk rot of corn to which the strains tested showed a marked differential in the amount of stalk rot that developed. The methods used in making inoculations and in taking data are described in detail.

The amount or extent of stalk rot following artificial inoculation is compared with the extent of stalk rot resulting from natural sources of infection in each of 13 dent corn single crosses. The correlations obtained in the comparisons are used as a basis for judging the efficiency of the artificial method of inoculation in measuring relative resistance.

The measurements of disease in the inoculated plants are (1) pith spread, and (2) cortical spread; and in the noninoculated plants are (1) natural infection, and (2) broken stalks. The correlation coefficients obtained between these various measurements for disease are as follows: pith spread and cortical spread, + 0.948; natural infection and broken stalks, + 0.909, pith spread and natural infection, + 0.853; pith spread and broken stalks, + 0.821; cortical spread and natural infection, + 0.878; cortical spread and broken stalks, + 0.899.

Data indicating the genetic or hereditary nature of reaction to stalk rot are presented.

It is concluded that relative resistance to Diplodia stalk rot can be measured by means of artificial inoculation with the fungus. Problems are mentioned, which need to be investigated, however, before the inoculation technique can be used as a tool in agronomic practice.

VARIETAL REACTION OF PEA TO A VIRUS FROM ALSIKE CLOVER

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(Accepted for publication March 14, 1938)

INTRODUCTION

In 1936 the writers reported a virus from alsike clover, *Trifolium hybridum* L., that was infectious to pea, *Pisum sativum* L. This virus was collected in the mountainous regions of northeastern Colorado, where it was commonly found affecting alsike clover.

This virus was shown by Zaumeyer and Wade[1] to be unlike those previously reported to be infectious to pea. Certain varietal studies were made earlier, but difficulty was experienced in securing sufficiently consistent percentages of infection for a significant determination of relative susceptibility or resistance. Since then, the use of carborundum powder as an abrasive in inoculating has made it possible to secure more consistent and dependable degrees of infection.

The purpose of this paper is to record the reaction of a number of pea varieties to this virus.

SYMPTOMS

The symptoms produced by this disease have been described earlier.[1] In general they resemble those produced by the common pea mosaic virus (*pea virus 3*).[2] With the exception of a few varieties such as Dwarf Telephone, Potlatch, Alderman and similar ones, no appreciable stunting of the plants is noted. A distinct mottling is characteristic of varieties such as Alaska, Dwarf Grey Sugar, Green Admiral, and Austrian Winter, while pronounced chlorosis is exhibited on the varieties Gradus, Stratagem, Prince of Wales, and Giant Stride.

METHODS

These studies were conducted under field conditions in Colorado, both in 1935 and 1936. Replicate plots of 111 varieties or strains of peas were grown each year. Fifty seeds of each variety (Table 1) were planted in each of 2 series, and the Broad Windsor bean, *Vicia faba* L., was systematically planted throughout the plot. This host is extremely susceptible to the virus and an excellent plant on which to rear the pea aphid, *Macrosiphum pisi* Kalt., which transmits the disease.

The virus inoculum was prepared by passing mosaic-infected alsike clover plants through a meat grinder. Carborundum powder was added to the expressed juice, which was slightly diluted with water. The inoculum so prepared was rubbed on the leaves of the plants by means of cheesecloth

[1] Zaumeyer, W. J. and B. L. Wade. Pea mosaic and its relation to other legume viruses. Jour. Agr. Res. [U. S.] 53: 161–185. 1936.

[2] Pierce, W. H. The identification of certain viruses affecting leguminous plants. Jour. Agr. Res. 51: (1935) [U. S.] 1017–1039. 1936.

TABLE 1.—*The reaction of varieties of peas to alsike virus 1*

Variety	Survival values:		
	1935	*1936*	*Avg.*
American Wonder (2)[a]	4.00	4.00	4.00
Ashford	4.00	4.00	4.00
Blue Bantam	4.00	4.00	4.00
Canner Gem (2)[a]	4.00	4.00	4.00
Delikatess	4.00	4.00	4.00
Dwarf Gray Sugar[b]	4.00	4.00	4.00
Excelsior, Nott	4.00	4.00	4.00
Excelsior, Sutton	4.00	4.00	4.00
Horal	4.00	4.00	4.00
Little Marvel	4.00	4.00	4.00
Perfection (2)[a]	4.00	4.00	4.00
Perfection, Renard	4.00	4.00	4.00
Perfection, Root Rot Resistant	4.00	4.00	4.00
Perfection, Wilt Resistant	4.00	4.00	4.00
Perfection, Wisconsin	4.00	4.00	4.00
Premium Gem (2)[a]	4.00	4.00	4.00
Short Admiral	4.00	4.00	4.00
Superb	4.00	4.00	4.00
Surprise	4.00	4.00	4.00
Wisconsin Early Sweet	4.00	4.00	4.00
Morse Market	3.95	4.00	3.98
White Eye Marrowfat[b]	3.85	3.85	3.85
Black Eye Marrowfat[b]	4.00	3.65	3.83
Horsford[b]	3.60	4.00	3.80
Hundredfold[b]	3.90	3.50	3.70
Progress	3.45	3.50	3.48
Laxton Superb	4.00	1.05	2.53
Chancelot	4.00	1.00	2.50
Hundredfold[b]	4.00	1.00	2.50
Dwarf Alderman[b]	2.85	1.80	2.33
White Eye Marrowfat[b]	3.65	1.00	2.33
Carter Eight Weeks	3.15	1.15	2.15
Champion of England	3.30	1.00	2.15
Maple[b]	3.10	1.15	2.13
Long Pod Alaska	3.10	1.10	2.10
Meteor	3.05	1.10	2.08
Progress	2.65	1.45	2.05
Lock Goil	3.05	1.00	2.03
Stella	3.00	1.05	2.03
Blue Bell	3.00	1.00	2.00
Bruce	2.80	1.15	1.98
Morse #200	2.95	1.00	1.98
Alaska	2.70	1.15	1.93
Lincoln	2.70	1.10	1.90
Phenomenon	2.15	1.65	1.90
Yellow Admiral[b]	2.00	1.75	1.88
Dwarf Alderman[b]	2.65	1.05	1.85
Early Eight Weeks[b]	2.60	1.05	1.83
Banqueter	2.70	1.05	1.88
Winner[b]	2.65	1.00	1.83
Thomas Laxton[b]	2.55	1.05	1.80
Maple[b]	2.50	1.05	1.78
Perfectah	2.45	1.05	1.75
Md. Alaska	2.35	1.05	1.70
Harrison Glory	2.25	1.05	1.65
Maple[b]	2.25	1.05	1.65
President Wilson	1.90	1.35	1.63
Sugar	2.20	1.05	1.63

[a] Numbers in parenthesis represent number of apparently identical strains.
[b] Other strains of the same variety gave different results.

TABLE 1.—(*Continued*)

Variety	Survival values:		
	1935	*1936*	*Avg.*
Duplex	2.05	1.15	1.60
Mammoth Melting Sugar	2.10	1.10	1.60
Senator[b]	2.00	1.15	1.58
Green Admiral[b]	2.00	1.10	1.55
Horsford[b]	1.95	1.15	1.55
Pride of the Market	1.95	1.15	1.55
Winner[b]	2.00	1.10	1.55
Bountiful	1.65	1.40	1.53
White Canada	2.00	1.00	1.50
Green Admiral[b]	1.80	1.10	1.45
Yellow Admiral[b]	1.85	1.05	1.45
Improved Hundredfold	1.85	1.00	1.43
Gilbo	1.80	1.00	1.40
Bluebell	1.65	1.10	1.38
Stratagem[b]	1.65	1.10	1.38
Green Giant[b]	1.70	1.00	1.35
Dwarf Gray Sugar[b]	1.50	1.15	1.33
Profusion	1.55	1.10	1.33
Alderman[b]	1.55	1.05	1.30
World Record[b]	1.50	1.10	1.30
Alderman[b]	1.55	1.00	1.28
Prince of Wales[b]	1.55	1.00	1.28
Rice No. 13	1.50	1.00	1.25
Swedish Yellow	1.50	1.00	1.25
V C	1.50	1.00	1.25
Abundance	1.45	1.00	1.23
Pioneer[b]	1.35	1.10	1.23
Potlatch	1.45	1.00	1.23
Stratagem[b]	1.40	1.00	1.20
Frost Resistant Stratagem	1.25	1.15	1.20
Ameer	1.25	1.05	1.15
Gradus[b]	1.00	1.30	1.15
Improved Pilot	1.25	1.00	1.13
Kootenay	1.05	1.15	1.10
Dairy[b]	1.00	1.10	1.05
Austrian Winter[b]	1.00	1.05	1.03
British Lion	1.00	1.05	1.03
Creole	1.00	1.05	1.03
Giant Stride	1.05	1.00	1.03
Gradus[b]	1.00	1.05	1.03
Tall Telephone	1.00	1.05	1.03
Daisy	1.00	1.00	1.00
Duke of Albany	1.00	1.00	1.00
Dwarf Quite Content	1.00	1.00	1.00
Edible Pod	1.00	1.00	1.00
Everbearing	1.00	1.00	1.00
G. O. P.	1.00	1.00	1.00
Late Gradus	1.00	1.00	1.00
Late Duke	4.00		
Onward	4.00		
Premium Gem	4.00		
Charles the First	3.95		
Carter Late Giant	3.75		
Extra Early	3.25		
Klevendon Wonder		4.00	
Lord Chancellor		4.00	
Black Eye Marrowfat[b]	2.90		
World Record[b]	2.25		

[a] Numbers in parenthesis represent number of apparently identical strains.
[b] Other strains of the same variety gave different results.

TABLE 1.—(*Continued*)

Variety	Survival values:		
	1935	*1936*	*Avg.*
Alderman[b]	2.20		
Thomas Laxton[b]	2.10		
World Record[b]	2.05		
Capucijner	2.00		
Mammoth Dwarf Sugar	2.00		
Maple[b]	2.00		
Prince of Wales[b]	2.00		
Yorkshire Hero	1.95		
Marrowfat	1.80		
Half Dwarf Melting Sugar	1.65		
Quite Content	1.65		
World Record[b]	1.65		
Senator[b]	1.55		
Austrian Winter[b]	1.25		
Mammoth Melting Sugar	1.25		
Admiral Beatty	1.00		
Moerheim Giant		1.10	
Early Eight Weeks[b]		1.05	
Green Giant[b]		1.05	
Japanese Pea		1.05	
Pioneer[b]		1.05	
Sutton Foremost		1.05	
Late Alaska		1.00	
Zwann's Paramount		1.00	

[a] Numbers in parenthesis represent number of apparently identical strains.
[b] Other strains of the same variety gave different results.

pads. Alternate plants in each lot were inoculated when about 6 inches high. The pea aphids that migrated to the peas and the Broad Windsor bean and multiplied there were not controlled. They never reached such numbers that direct aphid injury to the peas resulted, but they were responsible for much of the secondary dissemination of the disease.

A plant showing no symptoms at the end of about 10 weeks was recorded as healthy or resistant and given a rating of 4; mildly infected plants, 3; more severely infected, 2; and very severely, 1. No killing of the plants by the disease occurred. The products of the numbers of plants in each category, multiplied by the survival value assigned that class, were summed to develop a total survival value of each plot. Since the number of plants varied from plot to plot, depending upon the germination, occasional death from root rot caused by *Fusarium martii* var. *pisi*, and infrequent damping off due to *Pythium* sp., the total survival value of each plot was divided by the number of plants in the plot and this average was recorded as the survival value of the plot. Table 1 shows the values for the averages of 2 plots in 1935, 2 in 1936, and the 2-year average for those varieties observed in both years.

EXPERIMENTAL RESULTS

In Table 1, the varieties are arranged in order of their indicated resistance to *alsike virus* 1, based on the average of the 4 plots in 2 years. Where ties

occur the arrangement is alphabetical. The 34 varieties grown only one year are included in table 1, but they are not included in the ranking with varieties grown 2 years.

The varieties of peas tested were randomized within blocks and the statistical constants computed in a variance-analysis arrangement (Table 2).

TABLE 2.—*Analysis of variance of the reaction of pea varieties to alsike virus 1 for 1935 and 1936*

Variation due to:	Degrees of freedom	Mean square
Varieties	110	5.25[a]
Years	1	46.77
Blocks	1	.08
Blocks × varieties	110	.18
Years × varieties	110	.64
Error	111	.16
Total	443	1.65

[a] F = 32.81 when referred to error. 1 per cent point = 1.39.
S.D = 0.40.
SE_{m_4} = 0.20. Significant difference = 0.57.

The standard error of the mean of the reaction of 4 plots was found to be 0.20. For differences in reaction to be considered significant, they should exceed 0.57. In addition to the 111 pea varieties (Table 1) grown in 1935 and 1936, 24 others were included in 1935 and 10 in 1936. Variance analyses for 135 and 121 varieties, respectively, are shown in tables 3 and 4. The standard error of the mean of 2 plots is 0.40 for 1935 and 0.13 for 1936, with

TABLE 3.—*Analysis of variance of the reaction of pea varieties to alsike virus 1 for 1935*

Variation due to:	Degrees of freedom	Mean square
Varieties	134	2.59[a]
Blocks	1	0.15
Error	174	.33
Total	309	1.31

[a] F = 7.82 when referred to error. 1 per cent point = 1.27.
S.D. = 0.57.
SE_{m_2} = .40. Significant difference = 1.13.

TABLE 4.—*Analysis of variance of the reaction of pea varieties to alsike virus 1 for for 1936*

Variation due to:	Degrees of freedom	Mean square
Varieties	120	3.32[a]
Blocks	1	0.10
Error	120	0.0314
Total	241	1.67

[a] F = 105.73 when referred to error. 1 per cent point = 1.32.
S.D. = 0.177.
SE_{m_2} = Significant difference = 0.35.

significant differences of 1.13 and 0.35, respectively. In 1935 Potlatch was planted 15 times in each series and Alderman 7 times. The standard error of the mean of 30 plots of Potlatch is 0.10 and of 14 plots of Alderman, 0.15.

Twenty-four varieties of peas showed complete freedom from the disease in both years (Table 1). It is of interest to note that many of these are Gem-type peas, or rather closely related to this type. All strains of Perfection, including the wilt (*Fusarium orthocerus* var. *pisi*)-resistant and wilt-susceptible types, proved to be completely resistant to the virus except Perfectah, which showed a 2-year survival value of 1.75. Abundance, closely related to Perfection, had a survival value of 1.45 for 2 years. Most of the mosaic-free varieties in the group are canning types.

All of these varieties are market-garden types. Seven varieties were completely susceptible in both years, while others showed high resistance in 1935 and high susceptibility in 1936. Laxton Superb, Chancelot, one strain of Hundredfold, White Eye Marrowfat, and others gave decidedly more varied reactions in 1935 than in 1936.

The behavior of Laxton Superb is attributed to difficulties in interpretation of symptoms. Healthy plants are frequently light colored and slightly mottled, thus resembling those that are infected with mosaic. The Hundredfold strains proved to be of 2 different types, susceptible and resistant, with admixtures of types within a given strain. One strain of White Eye Marrowfat was found to be a mixture of resistant and susceptible biotypes. Chancelot was not available for further study, which made it impossible to determine the reason for variation in results.

The influence of environmental conditions upon the incidence and expression of the disease is shown by the much higher level of survival in 1935 than in 1936. The survival value of the 111 strains was only 74.47 per cent as great in 1936 as in 1935. The mean survival value for 222 plots grown in 1935 was 2.54, but in 1936 it was 1.89 for the same varieties. The difference amounts to the highly significant difference of 0.65 ± 0.038. In general, however, those varieties that ranked high in 1935 also rated fairly high in 1936.

DISCUSSION

As mentioned previously, the symptoms produced by *alsike clover virus 1* on peas are quite similar to those caused by the common pea-mosaic virus (*pea virus 3*). It would be difficult if not impossible to differentiate these 2 diseases on symptomological characters alone. Certain varieties of peas, however, react differently when inoculated with the 2 viruses. In this manner, together with other characteristic features that will be described in a later paper, the 2 viruses can be differentiated.

Although 50 per cent of the plants were inoculated artificially with *alsike virus 1*, the pea aphid, likewise, transmitted the virus to a considerable percentage of the remaining plants. Because of this, it is probable that a small percentage of the plants may have been infected with other viruses. It is believed that the percentage of plants thus infected was small, since, had

other viruses been present, it is probable that they would have infected a small percentage of the 20 varieties that showed complete resistance in both years (Table 1). Furthermore, the highest percentage of aphids transmitting the virus would, most likely, be infected with *alsike virus 1,* because of the abundant amount of inoculum present in the pea field, and not with other viruses that may have been present on other related hosts growing in proximity to the peas. Aphids from such plants would have been the main source of this infection, which is believed to have been negligible.

Most varieties that show resistance to *alsike virus 1* are likewise resistant to the common pea-mosaic virus. Blue Bantam, Surprise, and some strains of Dwarf Gray Sugar are resistant to *alsike virus 1,* but susceptible to the common pea virus.[3] Conversely, Morse's Market, Rice's No. 13, White Eye Marrowfat, and Hundredfold peas resist the common pea virus,[3] but are susceptible to *alsike virus 1,* with the exception of some strains of Hundredfold.

Alsike virus 1 is differentiated from the pea enation virus (*pea virus 1*),[5] the white-clover-mosaic virus,[6] and the virus from white sweet clover (*bean virus 2*)[7] by differences in varietal reaction of certain pea varieties. Nott Excelsior, Horal, Perfection, Surprise, Wisconsin Early Sweet and Hundredfold varieties resist *alsike virus 1,* but are susceptible to *pea virus 1.*[8] These varieties, together with Little Marvel, Premium Gem, and Onward, also resist *alsike virus 1,* but are susceptible to the white clover virus and the sweet clover virus, with the exception of Horal and Perfection, which are resistant to the latter. The virus recently described by Osborn[9] as the vein mosaic virus of red clover, differs from *alsike virus 1* in that Horal and Perfection are susceptible to this virus.

SUMMARY

Field studies were conducted in 1935 and 1936 to determine the varietal susceptibility and resistance of 145 varieties and strains of pea to *alsike virus 1.* The statistical constants were computed in a variance analysis. Twenty-four strains showed complete freedom of the disease in both years, while 7 were completely susceptible. Many of the resistant strains are of the Gem type or rather closely related to this type. Although the survival value in 1935 was of a higher level than in 1936, in general those varieties that ranked high in 1935 also rated fairly high in 1936.

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UNITED STATES DEPARTMENT OF AGRICULTURE,
WASHINGTON, D. C.

[3] Murphy, D. M. and W. H. Pierce. Common mosaic of the garden pea, *Pisum sativum.* Phytopath. **27**: 710–721. 1937.
[4] See footnote 3.
[5] See footnote 2.
[6] See footnote 1.
[7] Pierce, W. H. Viroses of the bean. Phytopath. **24**: 87–115. 1934.
[8] See footnote 2.
[9] Osborn, H. T. Vein mosaic virus of red clover. Phytopath. **27**: 1051–1058. 1937.

ROT OF MATURE TAP ROOT OF SUGAR BEET CAUSED BY PYTHIUM BUTLERI[1]

W. A. KREUTZER AND L. W. DURRELL[2]

(Accepted for publication March 5, 1938)

In the late summer of 1936 the senior writer had occasion to observe a peculiar root malady of sugar beets, *Beta vulgaris* L., in a large field in the Rocky Ford district. The disease was characterized by a wilting, yellowing,

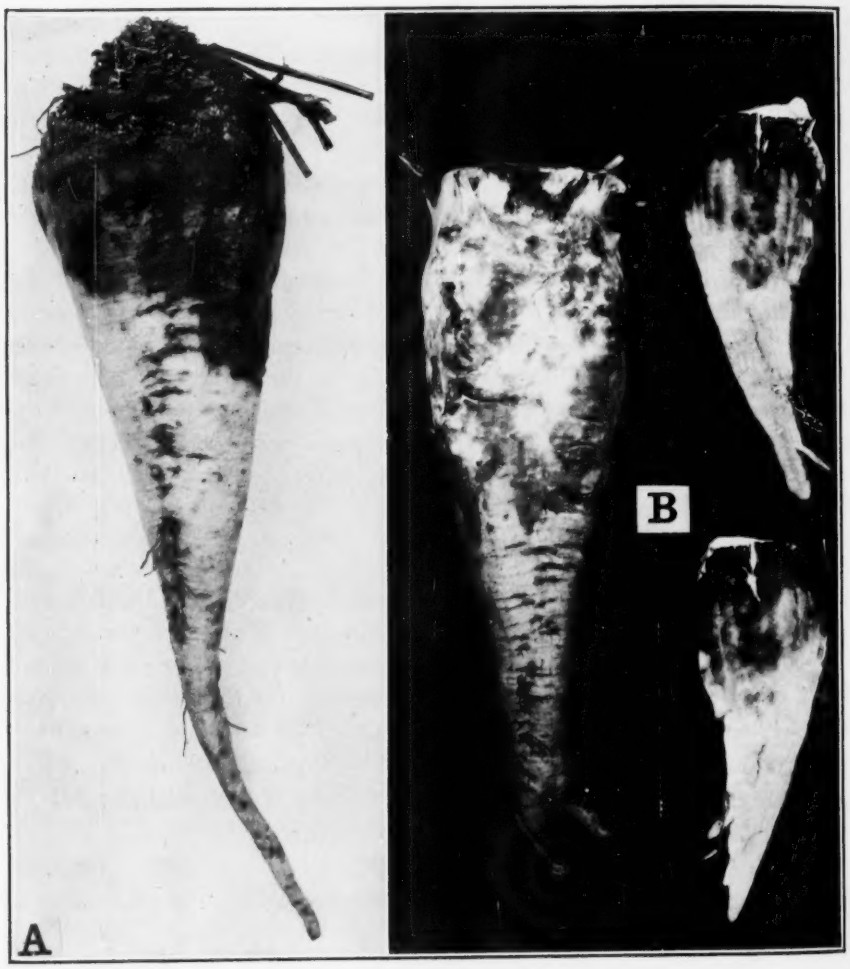

FIG. 1. A. Naturally infected field beet root showing the characteristic rot induced by *Pythium butleri*. B. Beet roots artificially inoculated with *P. butleri*. Note the mottled effect as shown by longitudinally cut roots.

[1] Published with the approval of the Director of the Colorado Agricultural Experiment Station.

[2] The writers wish to express their appreciation to Dr. Charles Drechsler for his identification and to Dr. W. J. Zaumeyer for his advice and assistance.

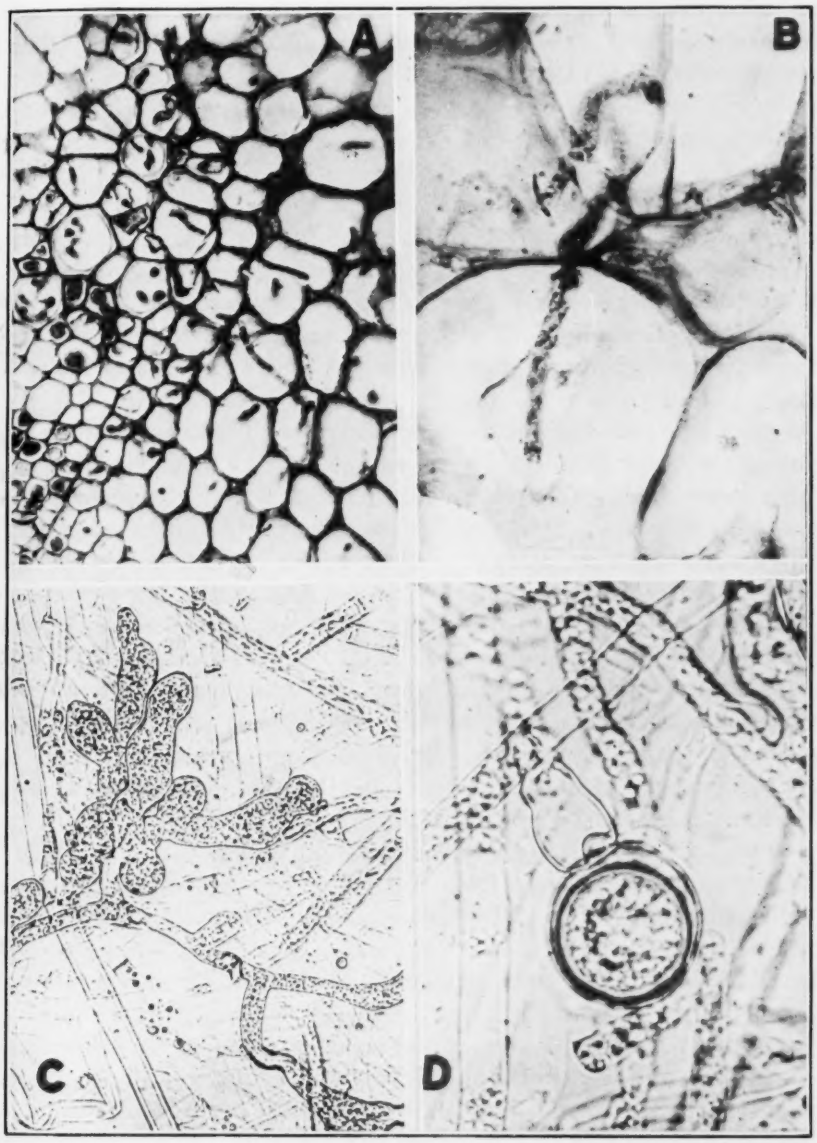

FIG. 2. A. Section through a vascular bundle of an infected root. Both the vessels and parenchyma show the presence of hyphae of *Pythium butleri*. B. Hypha of the parasite showing constriction at the point of cell-wall penetration. C. The elongated and lobulate sporangium of the fungus. D. Antheridium and oogonium of *P. butleri*.

and dying of the lower leaves of the plant that finally involved nearly all of the aerial parts. Affected leaves showed a brown, shrunken area extending from the base of the petiole toward the blade. Infected roots showed externally a deep brown discoloration, those portions nearest the crown being darkest, the color graduating toward the tip to a lighter shade, giving a dull mottled effect (Fig. 1, A). Internally, the roots showed a mummy-

brown discoloration (5) of those tissues attacked earliest. This discoloration graded off through a Dresden brown-Naples yellow to a Naples yellow in more recently invaded tissues (Fig. 1, B). The rot was of neither a soft nor a dry nature, the infected portions being relatively firm. Platings from the interior of affected roots yielded a pythiaceous organism, the pathogenicity of which was established by inoculations of and reisolations from numerous clean, injured and noninjured mature field-beet roots. In addition to the rotting of mature beet roots, the fungus was found capable of causing a severe damping off of sugar-beet seedlings. It was interesting to note that the virulence of the organism rapidly decreased in culture.

A histological study of affected root tissue showed the hyphae of the pathogen in profusion in all parenchymatous tissues and also in the vessels (Fig. 2, A and B).

Oogonia and antheridia of the fungus were produced readily when small pieces of artificially inoculated beet-root tissues were placed in dishes containing water blanks or Petri's solution (Fig. 2, D). Sporangia also were produced in this fashion, although they were more difficult to find than the former structures (Fig. 2, C). The best medium for sporangial production was found to be water blanks to which a small amount of greenhouse loam soil had been added prior to autoclaving. Oogonia from 48-hour-old water cultures averaged 24 μ in diameter, while the oospores averaged 20.5 μ. The organism was tentatively identified as *Pythium aphanidermatum* (Eds.) Fitzpatrick and cultures were sent to Dr. Charles Drechsler, who kindly consented to examine them. It is Dr. Drechsler's opinion that the fungus is one of the type generally referred to as *P. aphanidermatum,* but he considers that the binomial *P. butleri* is the proper one for the species in question on grounds set forth in an abstract in PHYTOPATHOLOGY (1).

Miestinger *et al.* (4) mention a root rot of beet in Austria caused to a lesser extent by *Pythium aphanidermatum,* and Simmonds (6), in 1933, reported the occurrence for the first time of a black rot of beet root in Australia induced by the same pathogen. Earlier, Edson (2) in this country, reported a fungus now generally considered as *Pythium aphanidermatum* (Eds.) Fitzpatrick, as a causal agent of damping off of sugar beet seedlings and capable of inducing injury to the feeding roots of half grown beets. No mention was made, however, of injury to the tap root proper. In his second paper (3) he described the organism as a new genus and species, *Rheosporangium aphanidermatus* Edson. Irrespective of whether or not the Rocky Ford fungus is considered as identical with the one previously described by Edson, the disease reported herein differs materially from that which he mentions.

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THE CONIDIAL STAGE OF HYPOXYLON PRUINATUM[1]

N. V. PONOMAREFF

(Accepted for publication March 7, 1938)

Povah[2] in 1924 was the first to describe the cankers caused by *Hypoxylon pruinatum* (Klotsche) Cke. and to suggest that the fungus was an important parasite on forest poplars. Lorenz and Christensen[3] in 1937 determined the amount of infection of *Populus tremuloides* by *H. pruinatum* in sample plots taken at random in the Lake States, and they state that "*Hypoxylon pruinatum* causes heavy losses in aspen throughout the Lake States and is probably one of the most important diseases of aspen throughout the range of the tree."

Despite the obvious importance of Hypoxylon canker, the organism has not been studied thoroughly. The perithecial stage has been known for some time, and the production of conidia by the fungus in culture has been observed, but the asexual spores produced in nature have not been described previously. A review of literature indicates that the conidial fructifications have been observed by several workers, but their true nature was not recognized.

Ellis,[4] in his description of a specimen collected in Iowa, states: "Surrounding the stromata and standing out obliquely like a coarse fringe, are short, coarse, bristle-like teeth, like the teeth of a Hydnum or Irpex. This curious growth also arises from the surface of the inner bark, for some distance around the stromata, soon throwing off the epidermis, and leaving the blackened surface of the inner bark exposed." On the basis of this character, he considered the fungus a new species and named it *Hypoxylon holwayii* Ellis. Ellis and Everhart[5] later decided that this fungus was within the range of variability of *H. pruinatum*. At this time they state: "The conidiiferous growth around the stromata may be only accidental, as it was not found in all the specimens."

[1] Paper No. 1617 of the Scientific Journal Series, Minnesota Agr. Exp. Station.
[2] Povah, A. Hypoxylon poplar canker. Phytopath. **14**: 140–145. 1924.
[3] Lorenz, R. C., and C. M. Christensen. A survey of forest tree diseases and their relation to stand improvement in the Lake and Central States. Mimeographed publication by the Bureau of Plant Industry, U.S.D.A. Washington, D. C., October 7, 1937.
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Povah[6] also may have seen conidial fructifications, as may be seen from his following statement: "In our specimens the bristly appearance is found commonly but not constantly. In some cases it is due to the presence of small perithecia protruding from the inner bark in which they are formed, little or no stroma being present. Sometimes the same spine-like appearance is obtained by the breaking down of the perithecia, leaving the jagged remnants of stroma and perithecial walls. In still other cases the dark teeth proved to be the more persistent parts of the inner bark left projecting after the softer part has been decayed."

The following account of the nature and development of the conidial stage of *Hypoxylon pruinatum* is based upon examination of material from numerous cankers developed naturally in the forest and of those produced as a result of artificial inoculations with cultures derived from ascospores and from conidia.

When the living bark of aspen trees is inoculated with *Hypoxylon pruinatum*, either naturally or artificially, the invaded cortex and the periderm above it first become light yellow. In a few weeks, due to shrinkage of the cortex, the cankered area is sunken below the general level of the bark and is split by many longitudinal shrinkage fissures; by this time the cortex has been partially destroyed and replaced by the black mycelium of the

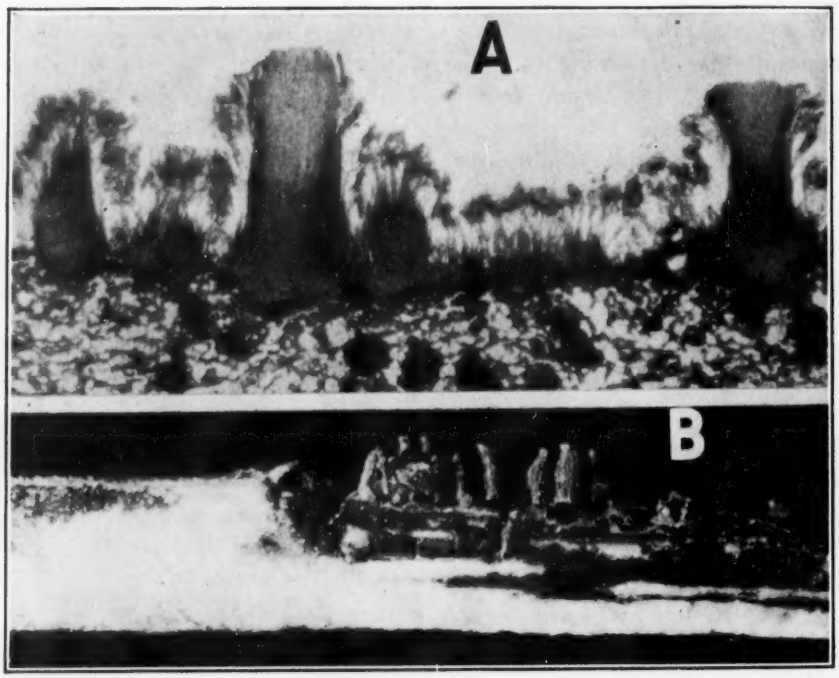

FIG. 1. A. Early stage in development of conidial stromata of *Hypoxylon pruinatum* on canker from natural infection. About × 85. B. Longitudinal section through cortex, showing group of old conidial stromata of *H. pruinatum* at the margin of an old canker. About × 15.

[6] See footnote 2.

fungus, which forms a subiculum on the surface of the cortex. About 3 months after infection the conidial fructifications begin to appear on this subiculum, still covered by the unbroken periderm. At first they are composed merely of rather compact, coremium-like tufts of branched conidiophores, as illustrated in figure 1, A, but they continue to increase in diameter and length and become more compact and stromatic in texture. The tips push out against the dead, thin, and brittle periderm, finally rupturing it and exposing the conidia. These stromata invariably have failed to develop on portions of the subiculum exposed by removing the periderm.

The bristly appearance of the conidial stromata is shown in figure 1, B. Due to weathering, and perhaps other causes, the stromata soon fall off, leaving only minute, jagged remnants and small circular scars that are clearly visible under a hand lens or binocular microscope, even on the surface of old cankers where perithecial stromata are present. The surface from which conidial stromata had been rubbed intentionally was compared with the surface of an old canker that bore the remains and scars of conidial stromata, and the appearance of the two was almost identical.

Two of the numerous cankers developed from inoculations made in June, 1937, were examined in October, 1937, and conidial stromata were found on one of them. They also were found on the borders of 15 3-year-old cankers developed from artificial inoculations on aspen trees at Cloquet, Minnesota, and on numerous older cankers of natural origin. By the time the perithecial stromata form, most, if not all, of the conidial fructifications have disappeared, and one could hardly recognize their remains without having seen them in a fresh and unbroken condition; hence it is not surprising that they have not been described previously.

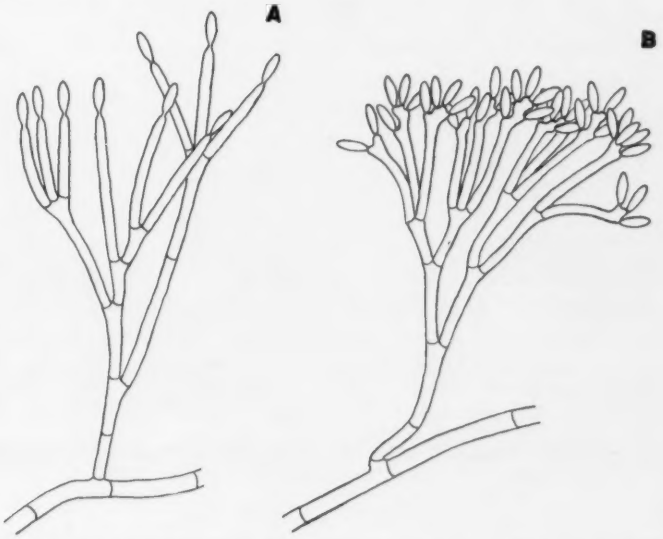

FIG. 2. A. Young conidiophore of *Hypoxylon pruinatum* in single-ascospore culture on malt agar. B. Old conidiophore of the same fungus.

The conidial stromata vary in basal diameter from 0.2 to 1.5 mm. and in length from 0.5 to 2.5 mm., averaging approximately 0.85×1.5 mm. The apex of a young stroma may be wider than the base, but the mature stromata always taper from the base toward the apex. The apices of those responsible for breaking the periderm are devoid of conidiophores, but, with this exception, conidiophores arise from the entire surface of both young and old stromata and of the subiculum. The conidiophores are 75 to 150 μ long, compound, the primary and secondary hyphae dichotomously branched, with either 2 or 3 terminal, spore-producing branches. At first, each branch bears a single terminal spore; but, later, the ends of branches are geniculate (Fig. 2, A and B). The conidia are hyaline, unicellular, fusiform to elliptical, measuring from 2×5.5–3×6.7 μ. They germinate readily on malt agar and produce one to several germ tubes. Cultures from conidia are within the range of variability of those from ascospores of *Hypoxylon pruinatum.*

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PHYTOPATHOLOGICAL NOTES

A Simple Method of Inoculating Wheat Seedlings With Paired Monosporidial Lines of Tilletia tritici and T. levis.—Studies of hybridization in *Tilletia tritici* (Bjerk.) and Wint. and *T. levis* Kühn necessitate the inoculation of wheat seedlings with paired monosporidial lines. The method described by Flor[1] has served the purpose thus far. He inserted the mycelium of 2 monosporidial lines into a wound at the base of each seedling. Hanna[2] and Becker,[3] likewise, used this method, and Becker stated that it was the best of 3 methods he tested. The writer[4] also used Flor's method but found it both tedious and time consuming, which limits its use for extensive inoculations. A more satisfactory method, since devised, is described below. This method is based on the principle that the secondary sporidia of *T. tritici* and *T. levis* are abjected forcibly from the sterigmata, as described by Buller and Vanterpool.[5] These workers used the same principle to inoculate seedlings with mass cultures.

A small piece of mycelium from each of the two monosporidial lines to be combined is transferred to a Petri dish containing potato-dextrose agar (1.5 to 2 per cent agar plus 1 per cent dextrose). The pieces of mycelium are

[1] Flor, H. H. Heterothallism and hybridization in *Tilletia tritici* and *T. levis.* Jour. Agr. Res. [U. S.]. **44**: 49–58. 1932.

[2] Hanna, W. F. The physiology of the fungi causing bunt of wheat. Proc. Fifth Pacific Sci. Congr., pp. 3195–3204. 1934.

[3] Becker, Theodor. Untersuchungen über Sexualität bei *Tilletia tritici* (Bjerk.) Wint. im Rahmen der Immunitätszüchtung. Phytopath. Zeitschr. **9**: 187–228. 1936.

[4] Holton, C. S. A new pathogenically distinct race derived from a cross between *Tilletia tritici* and *T. levis.* Phytopath. **28**: 371–372. 1938.

[5] Buller, A. H. R., and T. C. Vanterpool. The violent discharge of the basidiospores (secondary conidia) of *Tilletia tritici.* Buller, Researches in Fungi Vol. 5, 416 pp. Longman, Green and Co., New York. 1933.

planted on the surface of the medium near the edge of the dish, which is kept in an upright position until growth of the mycelium of both transfers has begun. The Petri dish is then turned on edge, with the side of the dish at which the mycelial colonies are growing at the top, and incubated in this position at a temperature suitable for development of the mycelium and secondary sporidia (10° to 18° C.). As growth continues the secondary sporidia, being forcibly abjected, fall downward, come to rest on the agar surface, and germinate to form mycelium, which produces more sporidia. Thus, a part of the agar surface becomes covered with sporidia and mycelial growth of the two cultures, as shown in figure 1. The culture is then ready

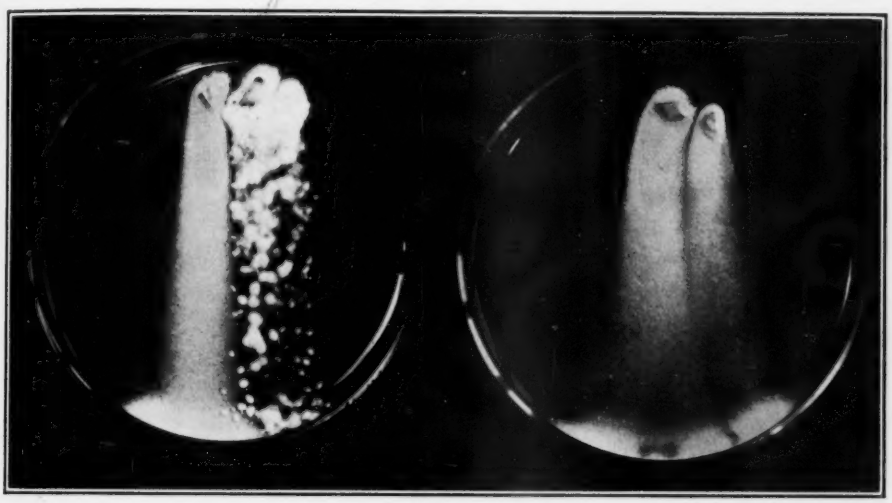

FIG. 1. Paired monosporidial lines of *Tilletia tritici* and *T. levis*, which were grown for inoculation purposes. Both pairs produced infection.

for use in inoculating the seedlings, and the Petri dish may be laid flat. The development of these cultures may require 2 to 3 weeks, depending upon the rate of growth of the monosporidial lines used. Greater coverage of the agar surface and a better mixture of the sporidia and mycelia of the two lines, than those shown in figure 1, may be obtained by transferring several pieces of mycelium of the two lines to one plate or by shifting the plates each day so that the sporidia spread in several directions instead of one.

The Petri-dish cover is then removed and inverted and the dish containing the culture also is inverted. Sterile filter paper is placed on the inside of the cover and moistened with sterile water. Fifty to 100 surface-disinfected kernels of a susceptible wheat variety are so placed on the moist filter paper as to be directly beneath the culture, when the inverted dish is put into the cover. The inverted Petri dish is incubated at 10° C. for 10 to 14 days, depending on the rate of growth of the seedlings. During this period secondary sporidia fall on the seedlings and germinate, thus exposing the seedlings to infection. When the seedlings grow larger they come into contact

with the sporidia and mycelium of the monosporidial lines above them, being thus further exposed to infection. The filter paper must be kept moist during the incubation period by adding sterile water from time to time. At the end of the incubation period the seedlings are transplanted to the greenhouse or field, either individually or as a group. The best results are obtained when the filter paper bearing the seedlings is lifted out of the Petri dish and all of the seedlings are transplanted in a group, still attached to the filter paper, without disturbing the roots, which is unavoidable when the seedlings are transplanted individually.

This method has the obvious advantage of permitting the testing of a large number of sporidial pairs and the inoculation of many seedlings in a short time without mechanical injury to the seedlings. Care must be exercised, however, to avoid interference by contaminating organisms while growing the inoculum and inoculating the seedlings. The gnarled and twisted seedlings are difficult to transplant, but this may be partly overcome by removing for a day the upper half of the inverted Petri dish before transplanting, to allow the seedlings to become erect.

Three experiments have been conducted in the last 2 years in which the inoculations were made by the above method, and high infection percentages were obtained each time. In the most extensive series of these inoculations, 26 of 74 combinations of monosporidial lines produced infections ranging from 1 to 57 per cent, with the majority above 20 per cent. It is very probable that still higher percentages of infection may be obtained with added experience and improved technique.—C. S. HOLTON, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, cooperating with the Washington and Idaho Agricultural Experiment Stations.

A Heritable Abnormality of Beans Resembling Mosaic.[1]—A leaf variegation in beans (*Phaseolus vulgaris* L.), the symptoms of which somewhat resemble a virus disease, was observed during an investigation of mosaic resistance. The symptoms differ from those produced by the common bean mosaic virus. Inoculation studies proved that this variegation was not caused by an infectious virus. Harrison and Burkholder[2] and Horsfall *et al.*[3] reported having found in the Wisconsin Refugee, a variety resistant to the common bean mosaic, plants that were apparently affected with a new virus disease. From the descriptions published by those workers, it is possible that the abnormal plants they observed are identical with the chlorophyl-

[1] Appreciation is expressed to F. J. Stevenson who offered valuable suggestions during the progress of the work and who assisted in the interpretation of the results, and to C. F. Andrus, who kindly permitted the use of certain data that were obtained from a number of segregating progenies used by him in a genetic study of bean anthracnose.

[2] Harrison, A. L. and W. H. Burkholder. U. S. Dept. Agr. Bur. Plant Indust. Plant Disease Reporter 20: 290–291. Oct. 15, 1936.

[3] Horsfall, J. G., W. H. Burkholder and O. A. Reinking. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Reporter 21: 318–319. Sept. 15, 1937.

deficient plants reported here. They are different from the two chlorophyll-deficient types reported by Parker.[4, 5]

Such plants were noted principally in hybrids in which Corbett Refugee was used as either of the parents. A study of a number of these hybrids by the writer indicated that the variegation was a heritable character. This character appeared in every varietal combination with Corbett Refugee but not in 100 per cent of all crosses involving each combination. This is evidence that Corbett Refugee is a mixture of biotypes.

The symptoms of this variegation vary considerably. The primary leaves may be practically devoid of chlorophyl and in such instances they frequently die. In other cases islands of green or streaks of yellow appear on the leaves. Such plants occasionally grow to maturity with the production of a few pods. Sometimes a plant may not show this defect until it has attained considerable size and then only a small amount of variegation is noted and on only a few leaves. Such plants grow to normal size.

When extremely variegated plants grow beyond the seedling stage, they are always stunted, with distorted leaves and pods and shortened internodes. They may also be rosetted. Very frequently defective plants manifest the variegation on only one-half of each leaflet accompanied by a curling of the affected leaflet toward the variegated portion, which may be the result of continuous growth of the unaffected cells accompanied by almost complete cessation of growth in the affected area. Pods produced on extremely affected plants are often curled, distorted, and smaller than normal.

The F_1 plants from reciprocal crosses of Corbett Refugee, which carries the gene for variegation, by other normal green plants of several varieties were green. The genetic constitution of Corbett Refugee is not entirely known. In the F_2 generation, a ratio of 15 normal green to 1 variegated plant was obtained from 6729 plants. The deviation from the 15 to 1 ratio was 0.46 which is 0.31 times the standard error. The reciprocal crosses in every case behaved alike.

The F_3 progenies of the green F_2 plants segregated into 3 classes, all green, 15 green to 1 variegated; and 3 green to 1 variegated. These three classes were approximately in the ratio of 7 : 4 : 4, respectively. The value of X^2 is 5.68 and P equals 5.8. The true breeding green progenies were slightly deficient while the segregating progenies were slightly in excess of the normal distribution. The variegated plants found in the F_2 generation did not breed true, a phenomenon that cannot be accounted for at present. Further studies of this variegated character and its genetic relationships are being conducted in an attempt to clarify the points in question.

Although the genetic hypothesis of duplicate genes does not explain all of the facts, the results show definitely that the abnormality is heritable and

[4] Parker, M. C. The inheritance of a yellow-spot character in the bean. Jour. of Heredity 24: 481–486. December, 1933.

[5] ———. Inheritance of a leaf variegation in the common bean. Jour. of Heredity 25: 165–170. April, 1934.

not the result of a virus infection.—W. J. ZAUMEYER, Bureau of Plant Industry, U. S. Department of Agriculture, Beltsville, Maryland.

A New Microcyclic Coleosporium on Limber and Piñon Pines.[1]—In the summer of 1937 Ivan H. Crowell collected an orange-red needle rust on *Pinus flexilis* and *P. edulis* in southern New Mexico. Macroscopically, the rust (Fig. 1, A) has the appearance of the peridermia of species of the genus *Coleosporium*. Microscopic examination of free-hand sections demonstrated, however, that the apparent peridermia were actually gelatinous telial horns composed of chains of teliospores (Fig. 1, B). The teliospores germinate at

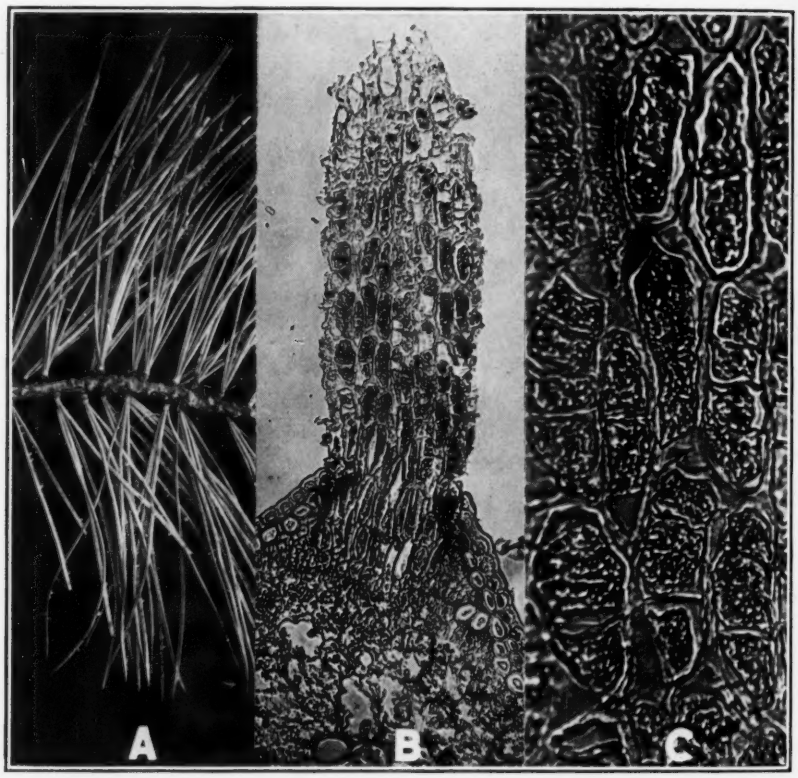

FIG. 1. *Coleosporium crowellii* on *Pinus*. A. Telial horns on *Pinus flexilis*. Approximately × 1. Photograph by Crowell. B. Free-hand section of a telium on *Pinus edulis* showing teliospores imbedded in the gelatinous matrix. The apical spores had germinated and disintegrated, the median spores were in process of germination, while the basal spores were unicellular. × 110. C. Magnified portion of B, showing the internal basidium. Sterigmata and basidiospores had not yet developed. × 510.

once with the formation of an internal basidium (Fig. 1, C). This immediate germination empties the spores in the upper one-half or one-third of the telial horn, so that the base is orange colored, while the apex is nearly colorless.

[1] Contribution from the Botany Department, Purdue University Agricultural Experiment Station, Lafayette, Indiana.

The rust is considered to be a microcyclic species of the genus *Coleosporium*, strongly simulating the aecial habit in size of telia and catenulation of spores, and it inhabits the genus *Pinus*, aecial host of the macrocyclic species of *Coleosporium*. A formal description of this rust, here named in honor of Dr. Crowell, follows.

Coleosporium crowellii, sp. nov. Pycniis non visis. Teliis amphigenis, subepidermalibus, gelatinosis, linguaeformibus, 0.3–0.7 mm, alt., 0.5–2 mm, lat., 0.1–0.15 mm, cr. (*Pini edulis*), 0.5–1.0 mm, alt., 0.1–0.3 mm, lat., 0.1–0.15 mm cr. (*Pini flexilis*) primo aurantiacis dein pallide flavis vel albido-flavis; teliosporis in catenas, cylindraceis, 17–24 × 50–65 μ, primo 1-cellularibus, dein ob germinationem 4-cellularibus, levibus, intus aurantiacis; basidiosporis globosis 14–18 × 16–20 μ. In foliis *Pini edulis* Engelm., Cloudcroft, Otero Co., N. M., July 3, 1937; Kingston and Hillsboro, Sierra Co., N. M., July 9, 1937; Wrights Cabin, Grant Co., N. M., July 9, 1937, *Ivan H. Crowell.* In foliis *Pini flexilis* James, James Canyon, Otero Co., N. M., Aug. 23, 1937, *Ivan H. Crowell* (Type). Type deposited in the Arthur Herbarium, Purdue University Agricultural Experiment Station.

The difference in the size of the telia from the two hosts is conspicuous, but in the absence of other differential characters, is not considered sufficient basis for a separation of the two collections.—GEORGE B. CUMMINS, Purdue University, Lafayette, Indiana.

Septoria brevispora (*Sacc.*) *Zeller renamed.*—The above name is untenable since Ellis and Davis used the same combination for a *Septoria* parasitic on *Bromus ciliatus* (Trans. Wisc. Acad. p. 99. 1903). I am therefore designating the *Septoria* on *Rubus* as **Septoria darrowii,** n. nom. (Syn. *S. brevispora* (Sacc.) Zeller) (Phytopath. **27**: 1002. 1937), in respect to Dr. George M. Darrow, U. S. Department of Agriculture.—S. M. ZELLER.